



1978

The Effect of Decalcified Allogenic Bone Matrix on the Healing of Periapical Lesions in the Rhesus Monkey

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Recommended Citation

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THE EFFECT OF DECALCIFIED ALLOGENEIC BONE MATRIX
ON THE HEALING OF PERIAPICAL LESIONS
IN THE RHESUS MONKEY

by

Bruce Kenneth Felder, B.A., D.D.S.

A Thesis Submitted to the Faculty of the Graduate School
of Loyola University in Partial Fulfillment of
the Requirements for the Degree of
Master of Science
May, 1978

DEDICATION

To my wife, Dawn, who graciously endured many long, lonely hours during the preparation of this thesis, and whose love, understanding, and support have contributed immeasurably to the fulfillment of a dream.

To my son, Jonathan, whose birth has allowed me to experience the joys of fatherhood, I hope this work will inspire you to far greater achievements.

ACKNOWLEDGEMENTS

To Dr. Franklin Weine, my advisor, my teacher, and my friend, I offer my sincerest appreciation for making the past two years the most intellectually stimulating of my professional career, and for allowing me to learn from the best.

To Dr. Marshall Smulson whose incredible dedication to teaching has helped to make Loyola a leader in the specialty of endodontics, your advice and thoughtfulness will never be forgotten.

To Dr. Hal McReynolds, thank you for sharing your knowledge and your time in helping with the evaluation of the histologic sections.

To Dr. Leon Coverly, your insights and suggestions have contributed significantly to the fruition of this project, and are truly appreciated.

I wish to express special thanks to my friends, and classmates, Dr. Dale Anderson, and Dr. John Gillan for their moral and logistical support. This project could not have been completed without their assistance.

I wish to extend my gratitude to the United States Air Force for making these past two years possible.

VITA

The author, Bruce Kenneth Felder, was born in Brooklyn, New York on the twenty-fourth of June, 1949.

He obtained his elementary education in the New York City public school system, and was graduated from Erasmus Hall High School, in Brooklyn, New York, in June, 1966.

The following September, he entered the University of Rochester, in Rochester, New York, from which he received the degree of Bachelor of Arts, with a major in psychology, in June, 1970.

He entered the University of Maryland School of Dentistry, in Baltimore, Maryland, in September 1970, and received the degree of Doctor of Dental Surgery, Magna Cum Laude, on the thirty-first of May, 1974. Upon graduation, he was elected to membership in the Omicron Kappa Upsilon honorary dental society.

In June, 1974, he entered active duty in the United States Air Force. His first year was spent at David Grant USAF Medical Center, Travis AFB, California where he completed a General Practice Residency program in June, 1975. For the following twelve months, he was stationed at Griffiss AFB, in Rome, New York where he practiced general dentistry. By competitive selection, he was accepted by the Air Force for sponsored training in the clinical specialty of endodontics.

In August, 1976, he entered the Loyola University School of Dentistry, where he began a dual course of study leading to the didactic de-

gree of Master of Science in Oral Biology and a Certificate of Specialty Training in Endodontics.

Starting in June, 1978, he will assume duties as Chief of Endodontics at the USAF Regional Hospital, Maxwell AFB, Montgomery, Alabama.

Dr. Felder is married to the former Dawn Marie Hollingsworth, of Sherill, New York, and has a son, Jonathan Louis.

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CHAPTER I

INTRODUCTION

The response of the periapical tissues to the toxic breakdown products of a non-vital pulp may vary from a slight widening of the periodontal ligament space to gross destruction and cavitation of alveolar bone. But, it is now well established that the vast majority of endodontic lesions will resolve after conscientious root canal therapy has been performed on the involved teeth (1-4). Special problems, however, can be associated with the larger of these lesions, especially following surgical intervention.

Garber (5) surveyed endodontic recalls and concluded that, in general, the larger the lesion, the longer it took to heal, with surgically treated cases requiring the longest time to resolve. McFarlane and Cina (6) noted that the larger operative defects remaining in the mandible subsequent to cyst removal were not amenable to the normal processes of healing which accompanied smaller defects. Thoma and Sleeper (7) claimed that bone cavities over 3 cm. in diameter could not reliably maintain a blood clot. Marble (8) noted that clot breakdown and infection were more likely to occur with a large lesion. Kovacs and Kerenyl (9) made a similar observation, stating that when a blood clot fills up a post-surgical cyst cavity greater than 2 to 3 cm. in diameter it undergoes retraction, leaving a dead space filled with serum. The changing pressure in the oral cavity may force saliva into the dead spaces through

the wound edges with resultant bacterial decomposition of the clot and healing complications.

These large lesions occasionally have been known to impinge on important anatomic structures, such as sinus cavities (Figure 1) or neurovascular bundles. Pathologic fracture can occur from weakening of involved bone, while prolonged healing time may result in delay of further reconstructive dental treatment.

Another problem associated with surgical treatment of large periapical lesions is the formation of apical scars (2, 10-13). According to Simon (14), a distinction should be made between healing and repair, both of which may occur upon resolution of periapical pathology. Healing or regeneration is the complete return of the tissues to their normal histologic appearance, and is the usual course of events, while repair represents the filling in of the defect with scar tissue. The apical scar is an excellent example of this phenomenon. According to Bhaskar (15), microscopically an apical scar shows relatively acellular, dense collagen bundles and represents an area where the healing process has terminated in the formation of fibrous tissue rather than bone. It may occur after surgical treatment when facial and lingual cortical plates are lost. Patterson, et al (2) claimed that apical scars can form without prior surgical intervention of periapical lesions.

The reported incidence of apical scars varies from 0.4 percent (16) to 2.5 percent (17) of periapical lesions. Although radiolucent, apical scars do not require further treatment and, for clinical purposes, the defect should be considered healed. Therein lies the problem. The

clinician viewing a periapical radiolucency (Figure 2), not knowing it to be an apical scar, is faced with a dilemma. Should he retreat the tooth or just keep it under periodic observation? What if the lesion is a cyst that has proven refractory to previous treatment? It is a well documented clinical fact that dental cysts expand progressively, and if untreated, may grow to an extremely large size (18, 19).

In view of the significant percentage of relatively large periapical lesions, and considering the clinical problems that they may present, it would be most advantageous for the clinician to have at his disposal an implantable material that would be capable of accelerating the repair of these lesions and inducing the regeneration of lost osseous tissue.

Hence, a study was undertaken to evaluate the effects of implantation of decalcified allogeneic bone matrix on the healing of pathologically created periapical lesions in Rhesus monkeys.

CHAPTER II

REVIEW OF RELATED LITERATURE

An enormous number of disease processes exist that manifest themselves by the formation of lesions and defects in the skeletal tissues of the body. Traumatic injury is another major cause of such defects. Virtually every division of the healing arts is confronted with the treatment of some type of bony defect. Approaches to the treatment of these problems have varied tremendously, but the basic goal has been the restoration or replacement of the missing tissues.

The body has a remarkable capacity to heal itself and osseous tissue is no exception, but the repair of large bony defects can take a long time, and thus efforts have been made to accelerate the process by the introduction of various types of implant or graft materials.

The ideal material for implantation into bony defects should have the following properties (10, 20-24):

1. It should be well tolerated by the host tissues and not provoke a foreign body or immunologic reaction.
2. It should stimulate or induce the formation of osseous tissue by host cells (osteoinduction).
3. It should permit the growth of new bone into itself (osteoconduction) and be easily revascularized.
4. It should be able to be resorbed in step with the regeneration of osseous tissue.

5. It should be easily shaped to fit the dimensions of the lesion.
6. It should be readily available.
7. It should be relatively simple to prepare.
8. It should be able to be sterilized.
9. It should be easy to store.
10. It should have a reasonably long shelf life.

Reports on bone implantation and grafting go back to the latter part of the 19th century, and between that time and the present, the volume of literature on the subject, as well as the variety of materials utilized is truly staggering. The basic categories, however, into which any material would fit are very simple (23, 135):

1. Autogeneic (autologous) materials: donor and recipient sites in the same individual.
2. Allogeneic (homologous) materials: donor and recipient are of the same species, but different individuals.
3. Xenogeneic (heterologous) materials: donor and recipient are of different species.
4. Alloplastic materials: materials made of a totally different substance than that of the recipient tissue. They may be biologically or non-biologically derived.

The literature review will be divided according to the above scheme, but an additional section will contain a discussion of the literature dealing with implants of decalcified tissue. Many of the articles to be discussed are concerned with several types of materials on a com-

parative basis. In such a case, the article is placed in the category that seems most appropriate.

1. Autogeneic (autologous) implants

There is virtual unanimity regarding the fact that autogeneic bone and marrow are the most effective materials to use for bone grafting (6, 8, 21, 24-40) since there is no problem with immunologic intolerance, viable cells may continue to live and aid repair, and donor material is always available. The main problem, however, is that the morbidity involved in obtaining the donor tissue, especially if large amounts are required, may overshadow the benefits to be derived, at least from the patient's viewpoint.

In 1913, Brown and Brown (41) investigated the osteogenic potential of periosteum in dogs. They implanted free periosteum, cortical bone with periosteum attached, and naked cortical bone into subcutaneous and muscle tissue, and bony sites. They concluded that bone would grow if it was placed in a functional location, regardless of whether periosteum was attached or not.

Phemister (42) reported much the same results a year later, but he also implanted boiled autogenous bone. The boiled bone implants resulted in far less osteogenic activity than the live implants. He noted that when fresh, vital bone was cut into small pieces before implantation, the results were superior to a single large piece. He concluded that more cells were able to survive because of the increased surface area available for nutrition.

In 1938, Levander (43) performed experiments to elucidate the mode

of origin of new bone formation. His results differed markedly from those of the earlier investigators. He believed that new bone was formed from mesenchymal tissue in the areas surrounding the graft. He felt that the specific stimulus took the form of a diffusible substance that was liberated from the graft and conveyed to the mesenchyme via the lymph. In his opinion, any viable transplanted cells would eventually die and contribute nothing to the repair process. To substantiate this, he injected alcoholic extracts of autogenous bone intramuscularly in 70 dogs. Cartilage or bone was induced to form at the injection site in 22 percent of the animals.

Mowlem (44) reported on 75 cases of cancellous autogeneic bone grafts to treat traumatic injuries of the face, jaws and limbs. He chose the ilium as the donor site because of the relatively high cellular content and porosity of the bone. The donor bone was fragmented to increase its surface area in order to create optimal conditions for survival of the greatest number of bone cells. He claimed no failures, with bony fusion demonstrable radiographically after two weeks and continuing until indistinguishable from normal.

In 1964, Bell (24) studied the resorption rate of various types of bone and bone substitutes implanted in the gastrocnemius muscle of dogs, since he believed that the success of a bone graft is partially dependent upon the rate of resorption. The materials compared were: autogenous bone, plaster of Paris, irradiated, non-irradiated and freeze-dried homologous bone, irradiated human bone, fetal and adult bovine bone, partially and completely deproteinized (anorganic) bone, and poly-

urethane foam. Although plaster of Paris resorbed the fastest, the autogenous bone was second fastest and, he felt, still the material of choice.

Hegedus (45), a Hungarian physician, reported the first known cases of periodontal bone grafting in 1923. At first he used oral donor sites but subsequently found it more advantageous to take the transplants from the tibia. He claimed considerable success with the procedure.

Cross (46-48) was one of the early proponents of bone grafting in the treatment of periodontal defects. He used several types of materials including intraoral autogenous bone, allogeneic deep-frozen banked grafts, and anorganic bovine bone. In a report of 68 implants (48), in which he used all three materials (one type per patient), he claimed demonstrable bone fill in 51 defects. Unfortunately, he did not make a separate evaluation of each type of material.

Linghorne and O'Connell (49), and Linghorne (50) were among the first to investigate autogenous grafting in periodontal defects from an experimental viewpoint. Fresh lesions (49) and chronic epithelialized lesions (50) were surgically created in the periodontium of dogs and filled with autogenous bone and dentin particles (49) and autogenous bone chips (50). Histologic evidence of osseous repair was more advanced in the implanted defects than the control defects. No essential differences were noted between grafts of bone or dentin. The investigators felt that the osteogenic effect of the grafts was due to the calcified intercellular material which when resorbed, provided some type of stimulus for the differentiation of connective tissue cells into osteoblasts.

Yuktanandana (40) performed essentially the same experiment as Linghorne (50) to determine if healing took place by reattachment or re-adaptation of periodontal tissues to the tooth root. Bone regeneration and connective tissue reattachment to the tooth was noted and was more advanced in experimental than control lesions. The amount of repair was inversely proportional to the severity and duration of inflammation.

Nabers and O'Leary (32) felt that the bone chips normally discarded during osteoplasty and osteoectomy procedures could provide an advantageous and readily available grafting material for intrabony periodontal defects. Eight cases were reported demonstrating marked decrease of pocket depth.

Robinson (35), realizing that autogenous material was the most effective for grafting, and believing that the donor material had to be completely resorbed before new bone formation could occur, reasoned that the particle size of the graft material was of major importance. He developed the osseous coagulum technique whereby bone dust is obtained from a suitable intraoral site, mixed with the patient's own fluid (blood and saliva) and implanted into periodontal defects. After re-entering treated defects at intervals up to two years, he concluded that the results were good and that the technique offered considerable promise as a routine procedure.

Coverly, et al (51) evaluated histologically the effects of the osseous coagulum technique on the healing of experimentally produced chronic periodontal defects in Rhesus monkeys. In the early stages of healing (one to four weeks), the coagulum-treated defects displayed a

more advanced level of repair than the control defects. They noted that the cortical bone fragments themselves did not proliferate, but apparently products liberated during their resorption induced competent host mesenchymal cells to differentiate into osteoblasts and form bone. They stated, "It is possible that the histochemical substances of the matrix of bone contain the precursor of the inducing substance." This is a very significant statement and will be discussed at length later.

Schallhorn (36, 37) believed that autogenous hematopoietic marrow was the most ideal implant material since it includes not only non-antigenic scaffolding, but also great numbers of viable cellular elements. His technique involved a two-stage procedure whereby marrow cores were obtained from the lateral or posterior iliac crest, deep-frozen, and then introduced into prepared periodontal defects. He has claimed dramatic results using this procedure, which have been demonstrated on surgical reentry. Despite these results, the considerable morbidity associated with obtaining donor material has limited its popularity.

McFarlane and Cina (6) have demonstrated rapid and complete regeneration of a very extensive multilocular cystic defect of the angle and ramus of the mandible with fresh autogenous iliac marrow which they feel is unquestionably the material of choice when rapid regeneration of a surgical cavity in bone is desired.

Bierly, et al (52) investigated simple refrigeration at 4°C as a storage technique for iliac biopsy cores in an attempt to circumvent the deep-freezing method of Schallhorn (36, 37) which required sophisticated equipment. The results indicated that between seven and fourteen

days, there is a precipitous drop-off in the ability of the cancellous bone and marrow to produce new bone. However, shorter storage periods resulted in osteogenesis comparable to that seen with fresh marrow cores.

Marciani, et al (53) undertook a pilot study to investigate the osteogenic potential of autogenous iliac crest marrow grafts in irradiated tissue. Three dogs were given 500 rads of Co^{60} radiation to the mandible twice a week for five weeks. Surgical defects were then created which were immediately filled with the graft material. Despite the large dose of radiation, bony union occurred in all mandibles with two of the animals exhibiting complete restoration of the defect at six months.

2. Allogeneic (homologous) implants

Allogeneic materials generally occupy an intermediate position with regard to desirability as bony implants. Host acceptance is usually less favorable than for autogeneic tissue, but donor material is available in relatively unlimited supply and its procurement does not involve additional morbidity for the recipient. Its power, in terms of bone induction, is highly variable and seems to depend as much, or more, on the method of preparation as it does on the nature of the material itself.

Cross (54) in 1957, reported on the histologic findings of a clinical case in which allogeneic bone chips from a bone bank had been implanted into a periodontal defect. Failure of the case due to a poor crown-root ratio necessitated extraction after three months. The tooth, together with the implant and surrounding periodontium, were removed

en bloc. Fragments of the grafted bone were seen with some new bone formation occurring around them. No osteoclasts or inflammatory cells were noted.

Kromer (29) discussed his experiences with clinical allogeneic bone grafting in over 100 cyst cavities, periodontal pockets and ridge defects. He used bone obtained from ribs removed in heart operations, resected femoral heads, and iliac crests. The donor bone was cut into pieces, washed in saline and stored in merthiolate solution. Of 30 periodontal defects grafted, 25 exhibited some decrease in pocket depth.

Gaffney, et al (55) attempted to increase the height and improve the contour of edentulous mandibular ridges in dogs with allogeneic rib bone preserved in acetone and formaldehyde. Their efforts were unsuccessful as all of the grafts either resorbed or sloughed.

In an effort to stimulate osteogenesis, Goncalves, et al (56) implanted allogeneic bone sticks, fixed in formaldehyde, into extraction sockets in dogs. The implants disturbed and retarded healing compared to control sockets.

Schaffer (57) implanted allografts and autografts of ear cartilage into surgically produced periodontal defects in Rhesus monkeys. Some implants were fresh, while others had been stored in 70 percent ethanol for varying amounts of time. The longer the cartilage remained in alcohol, the more rapid was its replacement by bone. No difference was noted between the allografts and the autografts. He stated that the implants appeared to serve two functions: they seemed to act as templates for bone growth, and as initiators of the differentiation of

mesenchymal cells into osteoblasts. Based on these apparently favorable results, Schaffer (58) and Schaffer and Packer (59) implanted nasal cartilage allografts in 70 periodontal defects in 26 patients. Sixty of the 70 implants resulted in new attachment.

Based on Schallhorn's previous success with autogenous marrow grafting (36, 37), Schallhorn and Hiatt (60) grafted cross-matched, frozen iliac allografts of cancellous bone and marrow into various types of periodontal osseous defects in 20 patients. The results compared favorably with those obtained with autogenous grafting except that two of the 20 patients developed antibodies to human lymphocyte antigens.

A considerable amount of work has been done with freeze-dried (lyophilized) allogeneic bone. The process basically involves deep-freezing the tissue and then placing it in a vacuum to draw off the frozen water. It allows convenient long-term storage, and it has been shown that the freeze-dried material may be reconstituted to its previous physical properties with any physiologically acceptable fluid such as normal saline, plasma, or even the patient's own blood (61).

Boyne (61) presented two cases in which freeze-dried allogeneic bone was implanted into dentigerous and radicular cystic defects. Replacement with apparently normal osseous tissue (as viewed radiographically) occurred in the relatively short period of three months. He claimed that this method of treatment might be superior to the traditional method of simple curettage.

Boyne and Lyon (13) compared the effects of three types of implant materials on the healing of periapical lesions of the anterior

teeth in nine patients. Each patient had similar multiple radiolucencies and thus, was able to act as his own control. Following one appointment root canal therapy and periapical curettage, one or more of the defects was implanted with either freeze-dried allogeneic bone, boiled defatted bovine bone, or anorganic bovine bone. Biopsy specimens were obtained by trephine or block section at four, five and eight months. The defects, control and implanted, which measured 5 to 8 mm. in diameter at operation, completely regenerated by five months. None of the defects, control or implanted, which originally measured 9 to 12 mm., had completely healed by eight months, but the implanted lesions exhibited more advanced healing. Although the implants appeared to have positively influenced healing, at least in the larger lesions, no single type of material stood out as being superior.

Boyne and Kruger (62) investigated the effect of five different implant materials on the healing of canine extraction sockets. The materials tested were: anorganic bovine bone, boiled defatted bovine bone, freeze-dried bovine bone, freeze-dried allogeneic bone and fresh autogenous bone. After twelve weeks, advanced healing was noted with the two types of freeze-dried bone and the autogenous bone. The other two types of implants apparently had little effect on repair.

Yeager and Boyne (63) found that there appeared to be a more favorable early acceptance of freeze-dried split rib allografts to the edentulous ridges of dogs if they were combined with autogenous hematopoietic marrow.

Hurt (64) surgically created chronic intrabony periodontal pockets

in dogs, filling half of them with freeze-dried allogeneic bone. After examining histologic sections obtained at four, six, ten and fifteen weeks, he concluded that the implants were well tolerated but failed to display significant osteogenic properties.

Marble (8) surveyed 91 clinical cases in which freeze-dried allogeneic bone was used as a graft material for cystic jaw defects. Forty-three of the cases represented radicular cystic defects. Follow-up times varied from three months to nine and a half years, with an average of 33 months. As determined clinically and radiographically, 82.4 percent of the grafts were successful. Failures were attributed either to placement of the bone chips in sites of existing infection or to inability to seal off the graft from contamination by oral, nasal or antral fluids. There was no clinical evidence of failure due to host rejection.

Spengos (38) treated 30 patients with large (over 3 cm.) cystic defects of the jaws, by surgical enucleation and implantation of freeze-dried irradiated allogeneic bone. Success was claimed in 73.4 percent of the cases, with follow-ups to two years. Failures were attributed to the same problems that Marble (8) noted.

Chalmers (65) investigated the immunological effects of fresh and freeze-dried bone allografts in rats. When fresh bone autografts were implanted in the paravertebral muscles of rats, they gave rise to vigorous new bone formation after four days. Fresh bone allografts were met with an initial inflammatory response which was followed by a delayed osteogenic response after four weeks. If the allografts were

freeze-dried, the initial inflammatory response was greatly attenuated and subsequent osteogenic activity increased. The lyophilization process appeared to inactivate histocompatibility antigens in the allogeneic grafts.

According to Curtiss and Herndon (66), and Curtiss, et al (26), the implantation of relatively small amounts of allogeneic, preserved bone is usually well tolerated by patients, whereas the introduction of massive allogeneic bone grafts has not met with the same success. They suspected that blood group antigens might play some role in allograft rejection and proceeded to test this hypothesis in dogs. The canine A factor blood group antigen was chosen for their experiment in which entire knee joints from A-positive dogs were implanted in A-negative dogs. In two of five recipient animals, low-titer agglutinins against the donor dogs' red blood cells developed. Immunologic tests on the sera of these two dogs suggested possible specificity of the antibodies for the canine A factor, but results were inconclusive.

3. Xenogeneic (heterologous) implants

Unaltered transplants of tissue from an entirely different species have routinely elicited unfavorable rejection responses in the recipient. For this reason, most such tissue has been processed in some way to render it less offensive to the host. The advantages associated with this category of implant material are essentially the same as those for allogeneic implants.

Orell (67) reported his clinical experiences with the surgical grafting of os purum, os novum, and boiled bone. Os purum was ox bone

prepared by a complicated physico-chemical procedure which freed the bone of lipids, connective tissue and some protein but still left some of the collagen matrix. It was used to fill various skeletal defects, and the author claimed that it was resorbed and replaced by host bone in two to three years. Os novum, essentially an autogeneic material, was made by implanting a slab of os purum subperiosteally against a patient's tibia for one to two months, by which time soft, new, vascular bone formed between the periosteum and the subjacent implant. This immature, vital bone was used in instances where extraskkeletal connective tissue separated two bones that were to be joined by transplantation, such as in spinal fusions. Orell used boiled bone to treat pathological bony conditions where proper shape and structure were of paramount importance. The procedure was essentially an autogeneic graft where the diseased bone was resected, boiled in saline, debrided of diseased tissue and replanted.

Forsberg (68) used finely ground, sterile os purum as an implant material for periodontal osseous defects. He showed eleven cases and claimed excellent results in one, satisfactory results in seven, and poor results in the remaining three cases after a post-operative period of up to twelve months.

Schaffer (69) implanted autoclaved human cementum and dentin shavings in surgical periodontal defects in a Rhesus monkey. Specimens were obtained at fourteen and 28 days. Surprisingly, no foreign body reaction and no resorption of the implant particles was seen. At 28 days, most of the implant particles had new bone on their surfaces.

Gilbertson and Clark (70) implanted Osteogen, a cultured calf bone paste, into extraction sockets of rats. The material resulted in moderate inflammation and a classic foreign body response. The experimental sockets were filled with new bone by 30 days whereas the control sockets had completely healed by ten days.

Beube and Silvers (71) placed a powder made from the long bones of sheep into surgically created defects in canine mandibles. They claimed accelerated healing, as seen grossly and histologically, compared to control defects after nine weeks.

Cross (72) presented a case report in which he reduced a 6 mm. intrabony periodontal defect to only 1 to 2 mm. after six months, by the implantation of cancellous bovine bone chips.

Gallie (73) stated that the sequence of events after implantation of autogenous grafts was first death of the graft followed by revascularization and finally resorption and replacement by host bone. With this in mind, he reasoned that since autogenous grafts lose any vital constituents that they contain upon implantation, boiled heterogenous bone should be able to be substituted with equally satisfactory results. He was apparently unaware of the immunological factors involved.

Beube and Silvers (12) used boiled bovine bone powder in fourteen cases including periodontal defects, periapical cystic defects, and extensive resorptive defects of edentulous ridges. They claimed a fair amount of success with this technique, which they attributed to the liberation of calcium salts from the fine, easily resorbed bone particles.

Beube, continuing his work with boiled cow bone powder, reported five cases of successful periodontal regeneration (74), accelerated repair of post-extraction sockets in three patients, and apicoectomy defects in nine patients (75). In the latter study, each patient treated had similar bilateral defects, one of which was filled with bone powder and the other left to heal as a control. Beube stated that the grafts hastened repair by about six months, compared to controls, in all cases. Again, he attributed the favorable results to a high local concentration of calcium salts.

In 1956, Losee (21) reported a preliminary study on the grafting of xenogeneic bone from which the organic fraction was totally removed by extraction with ethylene diamine. The resulting osseous product consisted of a porous, crystalline and non-crystalline inorganic lattice, and was termed "anorganic bone." According to Losee:

Other factors being equal, the speed with which acceptance, revascularization, absorption, and perivascular new bone formation takes place in a bone graft depends upon the ease with which osteogenic tissues of the host can penetrate and gain attachment to the graft. With removal of organic constituents, bone attains an enormously increased free surface area immediately available without the need for biological debridement.

The author made tibial defects in dogs which he filled with inlays of anorganic rat and bovine bone. Gross and histological evaluation after nine weeks indicated acceptance in all cases. Almost complete revascularization was noted as early as six weeks after implantation, and healing proceeded faster than in control defects.

Lyon, Losee and Boyne (76) implanted anorganic bovine bone into

exodontic defects in Rhesus monkeys and dogs. Post-operative examination after thirteen days demonstrated biologic acceptance and bony union.

Boyne, Lyon and Losee (77), encouraged by the favorable results in animals, implanted anorganic bovine bone into periapical, periodontal, and exodontic defects in 50 patients. The post-operative course was uniformly uneventful with host acceptance similar to that of the animal studies. After six to twelve months of observation of these patients, they concluded that it was possible to maintain alveolar ridge height and contour with this material.

Boyne (78), believing anorganic implants to be an excellent substitute for fresh autologous bone, continued his clinical trials of the material. Twenty-four grafts of anorganic bovine bone were placed into radicular cystic defects, periapical curettage defects, fissural cyst defects, and extraction sockets. Follow-up from eight to 28 weeks indicated host acceptance and excellent repair.

Hayward, et al (79) clinically employed anorganic beef bone to eliminate bony defects following various oral surgical procedures as a hemostatic agent, as a grafting material for the correction of micrognathia, and for the elimination of undesirable undercuts in alveolar ridges. They found the bone difficult to handle due to its extreme fragility, but it was well accepted by the host sites and appeared radiographically to result in bony regeneration.

Bell, et al (80) compared unprocessed bovine chips with anorganic bovine bone in extraction sockets in clinical patients. Periodic biopsies indicated that the raw bone chips elicited a foreign body

reaction. The anorganic bone chips resulted in far less inflammation.

Hurley, et al (28) used anorganic bone in various human surgical procedures including femoral fractures, cystic defects, spinal fusions, cranioplasties, and to fill cranial bur holes. They followed 149 patients from five months to two years. The implants were well tolerated and rapidly incorporated into a reparative callus at an early stage. They concluded, however, that the implants had little, if any, osteogenic power and that they basically prevented invasion of fibrous connective tissue, and served as a resorbable trellis upon which new bone could develop.

Boyne and Lyon (81) felt that a long-term histologic follow-up of anorganic bone implants was needed. Implants were placed in human periapical, periodontal and exodontic defects in fifteen patients. Biopsies were obtained from three to eighteen months later. They noted a general lack of osteogenic activity around the graft particles. They concluded that the persistence of unaltered particles for prolonged periods of time, and the general long-term histologic status of these implants indicated reevaluation of their clinical utility.

Toto and Giannini (82) investigated the osteogenic potential of anorganic bovine bone by implanting 40 mesh particles of the material subcutaneously in mice. The anorganic bone did not show any bone inducing properties after six months in situ.

Scopp, et al (83, 84) reported experimental and clinical work with a commercially available xenogeneic implant material called Boplant. It was derived from calf bone, and processing consisted of

detergent extraction, followed by chloroform and methanol extraction to reduce the lipid content, washing with sterile deionized water, sterilization by immersions in a liquid sterilizing agent, and finally lyophilization and vacuum packaging.

In the first report (83), Scopp, et al implanted the material into holes drilled in jaws of four Rhesus monkeys. After eight weeks, the grafts showed revascularization with osteoblastic activity and appositional bone growth. They concluded that Bopplant apparently stimulated osteogenic cells of the host to lay down new bone and appeared to stimulate new centers of osteogenesis apart from the graft, possibly representing fibroblastic metaplasia.

In the latter study (84), Scopp, et al reported on the use of Bopplant in the treatment of 77 oral bony defects including 54 intra-bony periodontal lesions, twelve apicoectomy defects and eleven furcal involvements. The patients were followed for up to twelve months. The implants appeared to be well tolerated, displaying no clinical antigenic effects. After twelve months, pocket depth was reduced by an average of 4 mm. They felt that Bopplant compared favorably with autogenous grafts.

Arrocha, Wittuer and Gargiulo (85) implanted Bopplant into premolar extraction sockets and alveolar defects in six dogs. The animals were given tetracycline intramuscularly to label new bone. Specimens were obtained from one to 180 days and evaluated with light and fluorescent microscopy. The results indicated the material to be well tolerated and that new bone formed around the grafted bone but at a rate com-

parable to bone formation in a similar wound without Boplant. The material seemed to serve as more of a core for bone to be deposited upon than as a stimulator of osteogenesis.

4. Alloplastic implants

The category of alloplastic implants includes any non-osseous material placed into a bony defect for the purpose of stimulating osteogenesis. It includes a very wide range of materials, both biologically and non-biologically derived, and is limited only by the imagination of the investigator and the tolerance of living host tissue.

Albee and Morrison (86) in 1920, reviewed the literature to date and reported that osmic acid, fibrin, blood, gelatin with lime salts, zinc chloride, thyroidin, glacial acetic acid, tincture of iodine, adrenalin, extract of hypophysis, copper sulfate, oil of turpentine, ammonia, lactic acid, silver nitrate solution, alcohol, carbolic acid, oak bark extract, vaccines, and sera had been used to stimulate bone growth without any appreciable success.

Thoma and Sleeper (7) utilized resorbable gelatin sponge as a bone substitute for large bone cavities.

Mandarino and Salvatore (87) claimed some clinical success with Ostamer, a polymer of rigid polyurethane foam, which they said had exhibited no tissue toxicity and encouraged host tissue to actively invade its lacunar-like framework.

Maccoomb, et al (88) also evaluated Ostamer. They tested its use in experimental fractures of long bones in sheep, dogs, and calves, and its effect on callus formation in healing fractures in rats. They found

it to be a non-toxic, distinctly inert material that could serve as a framework for the ingrowth of fibrous tissue, but they were unable to demonstrate any histologic evidence of osteoblastic activity within its lattice.

Dresser and Clark (89), and Moss (90) investigated Ivalon, a polyvinyl resin sponge. Histologic study of the implants in canine jaws revealed a minimal foreign body reaction and satisfactory acceptance by host tissue. The spaces within the sponge became filled with fibrous connective tissue, but very little bony ingrowth was noted.

Mitchell and Shankwalker (91) compared the osteogenic potential of calcium hydroxide with other materials, in the subcutaneous tissues of rats and in the tibiae of monkeys. Calcium hydroxide appeared to induce the formation of heterotopic bone in the rats, but a mixture of calcium hydroxide and gelatin seemed to delay the healing of the monkey tibial defects.

Shankwalker and Mitchell (92) studied the influence of calcium hydroxide and despeciated calf bone on the regeneration of surgical defects in the alveolus of dogs. Histologically, both materials were tolerated without any foreign body reaction. The despeciated calf bone had a slightly greater osteogenic influence after 158 days, but neither material proved to be particularly effective.

Ray, et al (93) implanted calcium phosphate and various types of auto-, allo-, and xenogeneic osseous materials into the anterior chamber of the eyes of guinea pigs and rats in order to observe their relative osteogenic potential. That particular graft site was chosen

because the fate of the grafts could easily be observed and the chance of spontaneous bone formation due to trauma alone was negligible. By six weeks, the calcium phosphate had evoked fibroblastic proliferation and an infiltration of plasma cells and giant cells, but no bone formation was seen at all.

Ray and Ward (33) compared synthetic hydroxyapatite with fresh and frozen autologous bone in surgical skeletal defects in dogs, cats and monkeys. It was concluded that synthetic hydroxyapatite crystals could be replaced by new bone but the material wasn't as effective as the autogenous grafts in repairing the defects.

Bahn (20) promoted plaster of Paris as a bone substitute since it appeared to offer a number of advantages including: simplicity, low cost, chemical stability, ready availability, ease of sterilization, excellent tissue tolerance, and rapid resorption and replacement by host bone.

Calhoun, et al (94) evaluated plaster of Paris as a bone substitute in surgically created mandibular fracture gaps in dog mandibles. Specimens were obtained at 60 and 90 days post-operatively. The experimental group showed more clinical, radiographic and microscopic evidence of bony union than did the control group without implants.

Kovacs and Kerenyl (9) implanted a coagulum consisting of fibrin powder, thrombin, venous blood and tetracycline into cystic defects of the jaws in a series of 85 patients. All lesions measured over 20 mm., and the majority were in the 30 to 50 mm. range. Clinically, the post-operative period was shortened, and radiographically, ossification was

accelerated compared to conventional treatment. The favorable results were attributed to the absence of dead spaces, the role of fibrin in the regenerative process and the antibacterial effect of the tetracycline.

Kulkarni, et al (95) discussed the possibilities of polylactic acid as a surgical implant. The cyclic diester of lactic acid can be polymerized to a material that is capable of being cast into any desired shape, or spun into fiber and woven. It is ultimately broken down by the body to lactic acid which is a normal intermediate of carbohydrate metabolism. It is unlikely that the material could elicit an immune response since it is not a protein and has no peptide bonds. When implanted into rats and guinea pigs it was found to be non-toxic and non-tissue reactive.

Nelson, et al (96) compared the osteogenic potential and tissue compatibility of biodegradable copolymers: polylactic acid/polyglycolic acid, and biodegradable tricalcium phosphate ceramic. These materials were implanted into surgical defects in the tibiae of rats, both in combination and singly, and evaluated at fourteen, 28 and 42 days. The ceramic resulted in uniform osteogenesis throughout the defect. The copolymer implants resulted in more gradual bone formation, progressing slowly from the wound peripheries. The two materials in combination behaved little differently from the copolymer alone. All implants were extremely tissue tolerant.

In recent years, considerable interest has been expressed in ceramic materials as osteogenic implants (30, 31, 97-104). There are two basic types: non-degradable (phosphate-bonded alumina), and biode-

gradable (different types of calcium phosphate). According to Bhaskar, et al (97), the non-degradable variety is perfectly tissue tolerant and permits ingrowth of connective tissue into its pores. Histologic sections through these implants have demonstrated that bone is deposited directly on the ceramic lattice. It does, however, have the disadvantage of being permanently retained in the tissues, which is a liability considering its lack of resiliency, low impact strength and poor fracture resistance. Biodegradable (resorbable) ceramics were developed for just these reasons.

Bhaskar, et al (97) implanted plugs of resorbable calcium phosphate ceramic in 66 rat tibial defects. Specimens were obtained for light and electron microscopy from three days to fourteen weeks post-operatively. The implants proved to be exceptionally well tolerated and tissue ingrowth was so rapid that it was impossible to grossly distinguish the implant from the surrounding bone after two weeks. With time there was a progressive decrease in the amount of ceramic with a corresponding increase in the amount of new bone. Structurally normal bone tissue was deposited directly on the ceramic. This study was not controlled and it was not possible to state if implantation of ceramic was preferable to leaving a defect unfilled.

Getter, et al (98) compared three types of biodegradable calcium phosphate slurries with regard to tissue reaction and osteogenic potential in 120 rat tibial defects. Tricalcium phosphate and calcium phosphate resulted in healing almost as rapidly as unfilled defects. Hydrated tricalcium phosphate was the slowest to resorb and defects containing it

took the longest time to heal. None of the materials produced any retrogressive tissue changes.

Cutright, et al (99) tested tricalcium phosphate ceramic in pellet form as a bone replacement material in tibial defects in 40 rats. Specimens were gathered from three to 48 days after surgery. The implants were very well tolerated, bone was deposited against and within the pellets, and marrow reestablished itself around and within the implants even before they had completely disintegrated. Degradation was approximately 95 percent complete by 48 days.

Driskell, et al (100) fitted resorbable tricalcium phosphate ceramic blocks into corresponding sized surgical defects in dog femurs. At eight weeks, normal tissue ingrowth was seen throughout the implant, most of which was calcified. The inflammatory response was even lower than with autogenous control grafts.

Mors, et al (101), and Mors and Kaminski (102) implanted biodegradable tricalcium phosphate ceramic implants into surgically created cleft palate defects in dogs. At six months, histologic evaluation demonstrated tissue-implant continuity without excessive granulation tissue, fibrous encapsulation, or sequestration. They concluded that the material appeared physiologically acceptable for bone implantation and did not seem to disturb normal growth of bone.

Levin, et al (30) created periodontal defects in all four quadrants of six monkeys and filled them with biodegradable tricalcium phosphate ceramic. By nine weeks, the defects had healed and the ceramic had not caused any deleterious tissue response.

Since autogenous iliac marrow is considered to be the most effective material for regeneration of lost periodontium, Levin, et al (31) decided to compare resorbable tricalcium phosphate with it. Chronic periodontal defects were created in six dogs into which either sterile ceramic implants or fresh iliac marrow were placed. One lesion per quadrant was left unfilled as a control. Specimens were obtained after one to 22 weeks and studied microscopically. The marrow-filled and the control defects healed faster than those containing ceramic, probably because the ceramic particles had to be resorbed before new bone could be formed, thus delaying the process. Despite somewhat more gradual healing, the authors felt that the results were encouraging since the ceramic displayed excellent tissue tolerance, resulted in complete regeneration of lost tissue, was simple to use, and was relatively inexpensive.

Biggs, et al (103) made surgical defects in the inferior border of seven rabbit mandibles and filled them with resorbable tricalcium phosphate ceramic implants which extended 2 mm. above the adjacent cortical surface. After 167 days, only five percent of the implant material remained, while the mass of new bone replacing it not only conformed to the original shape of the implant, but did so at a height greater than the original cortical level.

Bump, et al (104) tested a flexible, woven ceramic fabric as a graft material in the repair of surgically simulated intrabony periodontal pockets in two dogs and two monkeys. The woven ceramic was composed of zirconium oxide (ZrO_2) and yttrium oxide (Y_2O_3). Sections

were examined microscopically after thirteen and 26 weeks. The grafts exhibited outstanding biocompatibility and appeared to form a nidus upon which collagen and bone were deposited. Incorporation of the ceramic into osteoid indicated that the bone was definitely formed after the material was implanted. The woven ceramic was exceptionally easy to adapt to the irregularities of the bony defects and resulted in greater bone fill than in control defects.

Since collagen is ubiquitous in the animal kingdom, constitutes 20 to 30 percent of total body protein, and functions primarily as a supporting tissue and scaffolding for hydroxyapatite (105), it is only natural that it has aroused interest as a potential osteogenic implant material. The basic molecular unit of collagen, tropocollagen, is a triple helix composed of three similar polypeptide chains linked by hydrogen bonds. It is a rigid rod about 2800 Å long with a diameter of about 14 Å and a molecular weight of approximately 300,000 (106).

Mergenhausen, et al (107) implanted reconstituted rabbit skin collagen, in the form of a heat-precipitated gel, into the peritoneal cavities of rabbits and rats. Mineralization of the implants was observed after as short a period as sixteen days. Microscopic crystals were seen that were identified as hydroxyapatite.

Grillo and Gross (108) developed a method whereby cold, neutral solutions of collagen, isolated in dilute acetic acid from calf skin, could be cast as fibrous gels into any desired shape. The resulting material could be dried to a stable form which was readily sterilized and stored. The thermally reconstituted material was implanted into

heterologous tissues of rabbits and rats. A moderate fibrotic response was reported, with degradation of the collagen and replacement by host connective tissue. No calcification was seen. Immunologic response to the implants was evaluated and found to be minimal with respect to the collagen itself, but evidence was obtained for reactivity to contaminating plasma proteins.

Solomons and Gregory (109) implanted pepsin-treated citrate extracts of ox bone into artificially produced defects in the maxillae of 35 rats. Tetracycline injected intramuscularly was used to provide a quantitative evaluation of new bone formation by examining histologic sections under fluorescent microscopy and measuring their relative fluorescence. By ten days, the pepsin-treated collagen had accelerated tetracycline uptake eighteen times control values. The tetracycline content in experimental and control defects was essentially the same after 35 days. They concluded that the collagen implants had accelerated bone mineral deposition, and on that basis, felt justified in suggesting that it could act as a substitute for autogenous grafts.

Cucin, et al (25) filled rib gap defects in rabbits with specially prepared, non-antigenic calf skin collagen. The animals were sacrificed weekly from one to six weeks and radiographs were made of the implant and control defects just prior to death. The radiographs (read blind by a radiologist) showed significantly superior linearity in 64 percent and significantly superior apposition of segments in 82 percent of the implanted defects. The implants could not be grossly distinguished beyond three weeks and histologic examination showed that they

were invaded by fibroblasts and osteoblasts, and replaced by osteoid. No greater inflammatory response was noted than in control ribs.

DeVore (23, 110, 111), working with reconstituted collagen derived from calf skin, was able to produce different degrees of molecular cross-linkage of the material by treatment with different types of aldehydes. He implanted four such preparations, each cross-linked to a different extent, into surgically created defects in 143 rabbit mandibles (23). All of the grafts proved to be histocompatible with no signs of rejection. There was partial degradation of simple aldehyde cross-linked grafts, with new bone formation evident throughout, as early as four weeks which was complete by three to four months. The greater the degree of aldehyde induced cross-linking, the longer the time of degradation, with moderately cross-linked specimens not starting to degrade for six months, and highly cross-linked grafts intact and stable after as long as one year. In grafted animals, the mandible regained its original contour, while in control animals, defects remained after a year.

5. Decalcified tissue implants

The first account of the clinical use of decalcified tissue as an osteogenic implant material was reported by Senn (112) in 1889. He filled human bone defects with heterogenous bone that was previously treated with dilute hydrochloric acid. He stated, "Antiseptic decalcified bone is the best substitute for living bone grafts and the restoration of loss of substance in bone."

The following year, Miller (113) claimed that pieces of ox ribs

that had been decalcified with dilute hydrochloric acid and sterilized by soaking in dilute carbolic acid, appeared to be superior to fresh bone in obtaining repair of a human tibial defect.

Over half a century passed without any significant research in this area until 1957, when Ray and Hollaway (22) compared the rate of healing of standardized trephine defects in the parietal bones of rat calvaria following implantation of decalcified allogeneic bone matrix (DABM), frozen intact allogeneic bone, and frozen anorganic allogeneic bone. The bone matrix was prepared by decalcification in ten percent ethylene diamine tetraacetic acid (EDTA) at room temperature for twelve days, then washed with saline and stored at 4°C for three days prior to use. Specimens were collected at 42 and 43 days and viewed microscopically. The bone matrix was well incorporated into host bone, and vascular and osseous invasion of the implants and replacement by new host bone was much more extensive than that seen with the other materials. According to the authors, "Of the various materials used, it would appear that the best substitute for fresh autogenous bone grafts (if mechanical factors are not a consideration) is the organic matrix of bone devoid of its inorganic salts."

Young (114) essentially repeated the work of Ray and Hollaway, using rabbits instead of rats, to rule out a species variation in the response to the implant materials. Specimens were examined radiographically and microscopically after six weeks. He concluded that, in general, new bone formation was greatest in defects implanted with anorganic, and frozen whole allogeneic bone, and least in those filled

with DABM. In fact, he claimed that the decalcified implants may have delayed healing.

In 1961, Sharrard and Collins (115) used autogenous bone, decalcified in EDTA, in the surgical treatment of three scoliotic children. Biopsies of each graft site were made after six weeks, and the tissue examined microscopically. The decalcified autogenous bone was perfectly acceptable to the tissues and formed a good scaffold for the appositional growth of new reparative bone.

Heiple, et al (116) created standardized surgical gaps in the ulnae of fourteen dogs, and tested the ability of allogeneic bone, treated by seven different methods, to promote osteogenesis in the defects. Fresh, autogenous grafts were used as controls. Specimens were obtained from one week to one year post-operatively. The control implants were clearly superior, followed by: freeze-dried, frozen, EDTA decalcified, frozen irradiated, freeze-dried irradiated, fresh, and finally anorganic implants, in that order.

Freiberg and Ray (27) tested the effects of intact autogenous, intact allogeneic, DABM, and deproteinized allogeneic bone implants on the healing of surgically created femoral defects in 184 rats. Decalcification was accomplished in a saturated solution of dihydrogen-disodium versenate, pH 4.4, at room temperature. The animals were sacrificed after five to 184 days, and the specimens examined radiographically and microscopically. Control defects had completely filled by 20 days. The DABM implants were, without question, superior to the other allogeneic grafts. Connective tissue proliferation and invasion, and resorp-

tion and replacement by new host bone was only slightly slower than with the autogenous implants. The foreign body reaction seen around the intact allogeneic implants was not present, and 20 days after surgery, the margin between the host bone and the DABM implants was indistinct. The host tissues and the implant appeared to merge into one another, in contrast to the clear line of demarcation separating the host bone from the other types of implants.

All of the work discussed thus far with regard to the osteogenic potential of implanted, decalcified tissue has basically dealt with the subject from a strictly empirical standpoint. The "whys" and "hows" of the process have largely been ignored. However, Dr. Marshall R. Urist and his associates at the Bone Research Laboratory of the University of California at Los Angeles School of Medicine have been concerned with these very questions for over 25 years and must be regarded as pioneers in this important and fruitful area of research. Urist and his colleagues, since the early 1950's have published a considerable volume of material (39, 117-132) discussing their experimental findings with respect to the induction of bone by demineralized tissue implants, and the cellular interactions by which this phenomenon occurs.

Induction is a mechanism of cellular differentiation that depends upon the interaction of inducing cells and responding cells, as a result of which the latter assume a new pathway of development (117-119, 120, 123, 125). In the case of bone induction by dead, demineralized tissues, Urist uses the term "autoinduction" because the new bone originates

from a single source by reciprocal reaction between the inducing and responding cells, both of which arise from the same host bed (125).

Urist, et al (32, 118-132) have shown that a powerful substrate for interaction and differentiation of mesenchymal cells may be prepared from the matrices of hard tissues by decalcification in cold, dilute hydrochloric acid to preserve intercellular cement substance and minimize denaturation of protein. The substrate is so effective that if it is implanted in massive quantities in the musculature of the trunk and extremities of a rat, it can produce an amount of bone that is more than twice that which is normally present in the skeleton of the animal (127).

In 1965, Urist (118) published a summary of the results of 70 experiments on over 300 animals. Long bones excised from adult rabbits, rats, mice, guinea pigs, and dogs were cut into pieces and decalcified in cold (2 to 4°C) 0.6 N hydrochloric acid, and the resulting dead, demineralized matrix subsequently implanted into muscle, or bony sites in the same species of animal from which it was taken. Additionally, samples of human cortical bone obtained from accident cases or excised at autopsy were lyophilized and decalcified under similar conditions and implanted into bone defects in various skeletal system disorders in 21 patients. The overall percentage of positive results, in terms of induction of new bone in the host, was in excess of 90 percent.

Urist, et al (118, 120, 125, 130) found that the quantity of new bone produced is proportional to the available mass of inductively active

matrix and, provided that the mass of matrix available to ingrowing mesenchymal cells is a constant value, implants with relatively small and large surface areas yield equivalent quantities of new bone. Thus, grinding or pulverizing the matrix appears to offer no advantage and, in fact, may inhibit bone induction (126) if the matrix particles average less than 125 microns in diameter.

Bone formation occurs in intra- and extraskkeletal implants of decalcified matrix in the interior of excavation chambers or resorption cavities produced in the matrix by the action of connective tissue cells and sprouting capillaries (118, 120, 123, 125, 131). New osteoblasts are derived, not from elements of the donor tissue, but from proliferating, pluripotent (competent) ingrowing mesenchymal cells of the host bed. Urist's hypothesis of autoinduction makes the assumption that certain substances or degradation products of the dead matrix stimulate and attract wandering histiocytes, macrophages and giant cells to migrate into the interstices of the implant and form a "bone induction principle" (BIP). The BIP appears to be the product of the reaction between something in the matrix and the extracellular products of these migrating cells (120, 121, 123, 126, 129, 130). The BIP is the milieu for differentiation of mesenchymal cells into osteoblasts. Morphologically, the BIP consists of a network of new collagen fibrils and ground substance, but its specific chemical composition is not known. According to present concepts (129, 130), the acid-insoluble, three dimensional structure, particularly the cross-links, responsible for the weave

pattern of calcified matrices, emits the signal for the connective tissue cells that have taken up residence in excavation chambers, old Haversian canals, and interfibrillar spaces in the implant to synthesize and elaborate the BIP.

Since these wandering connective tissue cells are the first cells to contact the matrix, and thus the first to participate in the production of the BIP, they become the inducing cells. The induced or responding cells, which consist mainly of perivascular mesenchymal cells, are the osteoprogenitor cells and the first to differentiate. Once a bone induction system is established, one layer of induced cells then becomes the inducer for the next layer of responding cells (120).

Induction appears to proceed in two directions: centrifugally, to produce lamellar bone, and centripetally to produce new bone marrow cells. When the inductive substrate is implanted in soft tissue of mesodermal origin, such as into the belly of a muscle, the induction process continues until an ossicle is formed, complete with a central marrow filled cavity (117, 120, 121, 123).

Bone and bone matrix have been modified with various types and concentrations of chemical agents and radiation in order to determine the effects of these altered materials on bone induction and tissue tolerance.

Buring and Urist (122) exposed samples of decalcified bone matrix to graduated doses of Co^{60} gamma radiation, and implanted them into rat and rabbit muscle. The dose of radiation routinely used by bone banks

for sterilization of bone tissue (2 to 4 million rads), destroyed all inductive activity and thus limited the function of the graft to that of an inert scaffold. However, a smaller dose in the range of 0.2 to 0.5 million rads appeared to enhance bone induction since larger volumes of bone were produced, and at an earlier post-operative interval than with non-irradiated matrix. They attributed this result to reduced antigenicity of the resulting material.

Dubuc and Urist (121) stated that bone induction was eliminated if the matrix was exposed to heat above 80°C, cryolysis (repeated freezing and thawing), and treatment with various proteolytic enzymes. It was unaltered by fat solvents, salt solutions, or enzymes that extracted protein polysaccharides or lipoproteins from the matrix.

According to Urist (118), matrix decalcified with EDTA, mixed formic and citric acids or acetic acid produced osteogenesis in the same way as matrix decalcified with dilute hydrochloric acid, but EDTA produced a slightly lower percentage of positive results. Treatment with lactic acid failed to remove all the mineral, increased inflammatory reactions and decreased osteogenesis. Treatment with nitrous or nitric acid or with protein-altering chemicals impeded or prevented osteogenic activity in most cases.

Urist and Dowell (126) stated that concentrations of hydrochloric acid in excess of 0.6 N, or decalcification at room temperature for more than a very short time, destroyed all inductive activity in pellets of particulate matrix.

Urist, et al (120) found that lyophilization or treatment of the matrix with 70 percent ethanol greatly reduced any immunologic response and increased the yield of new bone.

Urist (127) noted that xenogeneic matrix was a poor bone inducer because the foreign tissue antigens resulted in ensheathment of the implant by a reticulocyte-plasma cell-lymphocyte barrier.

Urist (118) and Urist, et al (120) said that the principle difference between a graft of undecalcified allogeneic bone and an implant of hydrochloric acid decalcified allogeneic bone is immediately evident radiographically. An undecalcified bone graft is densely radio-opaque and is remodeled and replaced by less dense new bone relatively slowly over a period of months to years. Decalcified bone matrix implants are, at first, completely radiolucent but are replaced by low density new bone tissue very rapidly over a period of weeks to a few months. Apparently the decalcification process, by removing the mineral, makes the organic matrix accessible to the enzymatic activity of the invading connective tissue cells allowing the BIP to be synthesized and released (121, 125).

It has been shown that decalcified dentin and enamel, as well as bone, can exhibit osteoinductive activity. Bang and Urist (125) found that rat dentin decalcified in 0.6 N hydrochloric acid and sterilized in 70 percent ethanol, resulted in a high percentage of bone formation by four weeks after allogeneic implantation into muscle pouches in the ventral abdominal wall of rats. Histologically, the process appeared identical to

that observed with DABM. Xenogeneic decalcified implants of human and rabbit dentin were slowly resorbed. Undecalcified allogeneic dentin, sterilized in 70 percent ethanol also induced osteogenesis, but only after a lag phase of eight to twelve weeks.

Yeomans and Urist (123) grafted allogeneic dentin, bone, tendon, and muscle into abdominal muscle pouches, mandibular drill holes, and extraction sockets in rabbits. The dentin and bone were decalcified in 0.6 N hydrochloric acid and stored in 70 percent ethanol prior to use. Implants were excised at four, eight, and twelve weeks. Tendon and muscle did not produce any osteogenesis, but the decalcified bone and dentin did so in every case. However, the dentin matrix was resorbed and replaced more slowly than the bone matrix.

Huggins and Urist (131) reported an immensely important study in 1970. They implanted decalcified dentin subcutaneously in rats and obtained histologic sections after one to 365 days. The implants initiated a rapid mesenchymal chain reaction with bone and bone marrow forming by fourteen days and persisting until the end of the study. No inflammatory responses were observed in 100 consecutive grafts.

Urist (132) implanted allogeneic, lyophilized, decalcified enamel and dentin into intramuscular and intraosseous sites in dogs, rabbits, and rats. The enamel implants induced the formation of a bizarre mixture of cellular and acellular hard tissue resembling fish bone, while the dentin produced woven and lamellar bone and bone marrow. Urist concluded that the enamel matrix had bone histogenetic properties, while

the dentin matrix displayed bone morphogenetic activity.

Peterson (133) compared autogeneic and allogeneic decalcified dentin with fresh autogeneic bone in surgically created, large defects of the inferior border of the mandible in four dogs. Tetracycline was administered to identify new bone. The dogs were killed at twelve weeks and block sections of the graft sites were taken. The dentin grafts, both auto- and allogeneic compared very favorably with the bone grafts, clinically, radiographically, and histologically.

Bang, et al (10) cut standardized surgical defects, 7 mm. in diameter, into the region below the apices of the mandibular third molars in fourteen Java monkeys. Allogeneic decalcified dentin was placed into half, while the rest were left unfilled as controls. Specimens were obtained from one week to one year and examined microscopically. The dentin implants were well tolerated and induced osteogenesis and complete bone fill. The control lesions showed incomplete healing with persistent fibrous defects.

Knudson, et al (134) implanted sixteen allogeneic decalcified dentin implants into the gingiva of seven patients, and into the upper labial frenum in another eight. The implants remained in place from four to 30 weeks. They were well accepted by the tissues and showed evidence of varying degrees of resorption which was not related to time. There was no histologic evidence of bone induction in any of the implants.

Register, et al (34) prepared freshly extracted human teeth by

sectioning, removal of all enamel, cementum and pulp, and decalcification in 0.6 N hydrochloric acid at 2°C for five days. The dentin matrix was then lyophilized, treated with 0.2 million rads of Co⁶⁰ radiation and stored in 70 percent ethanol for up to one year. Two periodontal patients volunteered to have the dentin matrix implanted into their gingiva through small stab wounds. Specimens were obtained for study at 45, 60 and 90 days. All implant sites healed within a week and no clinical evidence of immunologic reaction was noted despite repeated implantation. Differentiation of mesenchymal cells to osteoblasts was seen by 60 days with osteoid formation and occasional mature ossicles. At 90 days, mature marrow-filled ossicles were clearly demonstrable. Only where the implant had communicated with a periodontal pocket did bone induction fail to occur.

Nordenram, et al (135) used allogeneic decalcified dentin to fill cystic jaw defects in 33 patients. A second group of 37 patients with similar lesions, treated by enucleation only, were the controls. The observation period ranged from six to 66 months. The implanted group appeared to have somewhat more severe post-operative symptoms initially, but exhibited a lower incidence of residual defects and a higher incidence of complete healing of the cyst cavities on radiographic examination.

Morris (136) decalcified human tooth roots, with cementum intact, in 0.6 N hydrochloric acid at 4°C and subsequently implanted them as xenogeneic grafts into the abdominal subcutaneous tissues of 12 rats.

The animals were killed after one week to one year and histologic sections of the implants subsequently examined. The tooth implants showed resorption and invasion by connective tissue cells and even partial recalcification, but no bone formation was noted in any specimen. The author claimed that his results were in direct disagreement with those of Huggins and Urist (131), but he should not have expected any significant display of bone induction using a xenogeneic substrate.

Narang and coworkers (137-145) have performed extensive animal experimentation using decalcified allogeneic bone matrix (DABM) in numerous types of bony defects and implant sites in an effort to demonstrate conclusively the exceptional osteoinductive power and tremendous clinical potential of the material in the areas of oral surgery, periodontics and endodontics.

Narang, et al (137) prepared standardized surgical defects on the buccal surface of the mandible of four dogs. Some defects were left to heal with a blood clot while the rest were filled with either fresh autologous bone or DABM. The animals were killed to provide two, four, six, and eight week specimens. The control defects showed a gradual increase in bone formation but had not healed completely by eight weeks. The autologous grafts showed extensive bone formation but still displayed fibrous tissue between the implant and the graft at eight weeks. The DABM grafts showed the most advanced healing at eight weeks with almost complete bone fill including Haversian canals and lamellae. There was no sign of graft rejection.

In a study involving three different species, Narang and Wells (138) implanted cortical and cancellous DABM subperiosteally against the edentulous ridges of dogs, against the buccal plate of the mandible in dogs and monkeys, and against intact fibulae in rats. Tetracycline was used to label new bone. The animals were killed from one to eighteen weeks after surgery and histological and ground sections were examined. All of the cortical grafts healed well and all of the cancellous grafts were exfoliated. Bony union between the host bone and the cortical DABM grafts was noted and appeared histologically similar in all of the onlay grafts in all three species.

Narang, et al (139) created standardized fibular fracture gaps in rats. Some of the gaps were filled with DABM, others were filled with decalcified xenogeneic canine bone fragments, and the rest were left unfilled. By fourteen weeks, the DABM grafts resulted in complete bony union, while the control defects had filled in with muscular and connective tissue but no bone. Fifty-five percent of the xenografts were rejected the first week, exhibiting a purulent exudate and sequestration. Those that were not rejected, induced only minimal amounts of bone. There was no clinical evidence, whatever, of rejection of the DABM grafts.

Narang and Wells (140) surgically produced interproximal and facial periodontal defects in 27 dogs. The defects in 77 quadrants were filled with DABM, while the rest were left unfilled. New bone was labelled with tetracycline. The animals were killed after one to 21 weeks.

Bony union was noted between the DABM implants and the host tissue by eight weeks, and bony union between the new bone and the cementum surface was seen after thirteen weeks, apparently resulting in ankylosis. The control sites exhibited considerably less bone fill. The DABM remained well tolerated by the host tissues.

Narang, et al (141) extracted all of the maxillary and mandibular premolars in six dogs, and after radical alveolectomies, allowed the wounds to heal. The sites were reexposed after six weeks and half received DABM subperiosteal onlays. The animals were killed after sixteen weeks. The DABM grafts resulted in bony union and increased height and width of the ridges compared to the controls. There were no adverse tissue reactions noted in any of the grafts.

Narang and Wells (142) tested the regenerative potential of DABM to correct experimentally made, extensive defects in the inferior border of the mandible in nine Rhesus monkeys. Half of the defects received the implants while the rest served as controls. New bone was labelled with tetracycline, and specimens were obtained after eight, nine, eleven, and thirteen weeks. The implanted defects healed completely and normal bony contour was reestablished. The controls displayed incomplete bone fill at thirteen weeks. No trace of immunologic rejection of the implants was seen and the authors suggested that the process of decalcification might have rendered the matrix antigenically neutral.

Narang and Wells (143) created fresh palatal defects in six monkeys and chronic palatal defects in seven dogs. All were filled with

DABM. Tetracycline was used to label new bone and specimens were obtained after three to twelve weeks. Clinical, histologic and fluorescent studies revealed that the grafts were rapidly resorbed and replaced by new bone and marrow, developing a firm bony union. There was no evidence of acute or chronic inflammation in any of the implant sites. The results suggested that DABM grafts might have a place in the treatment of cleft palate patients.

The ability of DABM to enhance new bone formation in experimental apicoectomy defects was investigated by Narang and Wells (144) in 1973. Thirty-one anterior teeth in three dogs and two Rhesus monkeys were apicoectomized after conventional root canal treatment. The periapical defects of fifteen teeth received DABM implants trimmed to fit, while the remaining sixteen were left unfilled. Radiologic and histologic examination at seven and eighteen weeks indicated more abundant new bone formation in the implanted defects. In a related study, Narang and Wells (145) implanted DABM into apical root-end preparations in dogs and monkeys in an attempt to obtain a biologic seal. Bone around the apices of 48 anterior teeth was removed, the apical one-fourth of each root was sectioned and a small cavity preparation made which was filled with tiny pieces of DABM. In another fifteen teeth, apicoectomies were performed but the defects were left unfilled. Root canal therapy was not performed on any of the teeth. Microscopic study revealed formation of new bone and cementum in the bone lesions and root-end preparations of the implanted areas within eight weeks. The control defects demonstrated

chronic inflammation and necrotic tissue with very little new bone, after twelve weeks. The studies did not simulate the clinical situation because the bone lesions were artificially created and the environment in such lesions is different than in chronic inflammatory defects.

Throughout all their studies, Narang and his associates stressed the many advantages of DABM as an osteogenic implant material. Host acceptance of the material was exceptionally good. DABM appeared to be a powerful inducer of new bone formation. Procurement of adequate amounts of material was never a problem. Preparation and sterilization were easily accomplished, and long-term storage in 70 percent ethanol did not diminish the inductive potential of the material to any noticeable degree. Its relative softness and pliability allowed simple and accurate trimming of the material to fit the irregularities of virtually any bone defect. The additional trauma of an extra operative site to obtain donor tissue was eliminated. For these reasons, it appears that DABM approaches the qualifications for a clinically acceptable material for bone implantation as outlined at the beginning of this chapter.

CHAPTER III

MATERIALS AND METHODS

Four healthy, adult Rhesus monkeys (Macaca mullata), three females and one male, weighing 4.0 to 8.4 kilograms, were used as animal models to evaluate the effect of decalcified allogeneic bone matrix (DABM) on the healing of periapical lesions. The animals were procured, treated and maintained at the Loyola University Medical Center Animal Research Facility. They were individually caged in a well ventilated, well lit room devoted exclusively to primates. The monkeys were under continuous veterinary supervision, and fed a nourishing diet consisting of standard laboratory chow, fresh fruit, and water ad libitum.

Prior to the start of each operative session, the animals were given intramuscular injections of phencyclidine hydrochloride*. A standard dosage of 0.6 cc. (12 mg.) tranquilized them sufficiently, within 20 minutes, to allow their safe removal from the cages.

At the same time that the tranquilizer was administered, the monkeys also received subcutaneous injections of 1.0 cc. (0.5 mg.) of atropine sulfate** which significantly reduced their oral secretions and facilitated the operative procedures.

*Sernylan, Bio-Ceutic Laboratories, Inc., St. Joseph, Missouri

**Atrosed, Burns-Biotec Laboratories Division, Chromalloy Pharmaceutical, Inc., Oakland, California

When anesthesia of surgical depth was required, the animals received intravenous injections of pentobarbital sodium*. Venipuncture was made on the dorsal surface of the lower leg after shaving the skin with electric shears. The barbiturate was slowly administered until adequate anesthesia was obtained as determined by deep, regular breathing and loss of the eyelid reflex. The average dosage needed to achieve this level was between 1.5 and 2.0 cc. (100 to 130 mg.), and usually provided one and a half to two hours of working time. Occasionally, additional anesthetic was required due to the length of the procedure.

Radiographs were taken on each animal at several points in the study. A hand-held laboratory x-ray machine was used (Fig. 3), with settings of 60 kvp. at 20 ma., and an exposure time of 0.4 seconds. Standard sized dental periapical films** were used, which were developed and fixed for 20 and 40 seconds, respectively, in rapid processing solutions*** in a portable table-top developing box.

The experiment was divided into two distinct phases: 1) pulpal inoculation with a pure culture of Streptococcus faecalis in order to produce periapical lesions in the animals, and 2) endodontic treatment of the infected teeth followed by periapical surgery and implantation of DABM into the periapical areas of selected teeth. The second part followed the first by eleven to 22 weeks.

*W. A. Butler Company, Columbus, Ohio

**Size 2, DF-58, Eastman Kodak Company, Rochester, New York

***Insta-Neg/Insta-Fix, Microcopy, Culver City, California

Phase one

Root canal cultures were taken from the teeth of routine clinical patients treated in the Graduate Endodontic Clinic of Loyola University Dental School.

The root canals of the teeth to be cultured had been cleansed and shaped during one or two previous visits. At the culture appointment, the tooth was isolated with a rubber dam and the operative site disinfected with 95 percent ethanol applied to the area on a cotton swab. A sterile bur in a high speed handpiece was used to remove the temporary filling to gain access to the root canal system. A sterile paper point was grasped in a sterile cotton pliers, inserted into the root canal, and left in place for 30 seconds. Upon removal, the paper point was immediately transferred to a tube containing thioglycolate medium which was flamed, capped, and placed in an incubator set at 37°C. A total of nine cultures were taken from the teeth of as many patients. Three were visibly positive within 48 hours, while the rest were negative after a week, and discarded.

Bergey's Manual of Determinative Bacteriology (146) was consulted for the proper bacteriologic testing procedures to isolate the Streptococcus faecalis organism. One of the three positive cultures proved to contain the desired organism after employing the following series of tests:

1. Growth on blood agar yielding small, round, raised, milky colonies, fairly abundant and confluent after streaking.

Hemolysis is variable. It may be alpha (greening) or gamma (none).

2. Growth in nutrient broth containing 6.5 percent sodium chloride.
3. Growth after heating a freshly inoculated tube of nutrient broth in a water bath at 60°C for 30 minutes.
4. Growth on nutrient agar containing 0.1 percent methylene blue.
5. Growth in nutrient broth adjusted to pH 9.6.
6. Growth on nutrient agar incubated at 10°C and 45°C.
7. Fermentation of sorbitol.
8. Growth in S-F selective media demonstrating a colorimetric change from red to brown.
9. Gram staining, yielding Gram-positive, large, ovoid cells seen in pairs or short chains.

Once isolated and identified, the organism was maintained on refrigerated nutrient agar plates. Fresh plates were inoculated weekly until the organism was needed for the experiment, at which time a tube of brain-heart infusion broth was inoculated and incubated at 37°C eighteen to 24 hours prior to use in the animals.

The monkeys were removed from their cages, weighed and brought to the operating room where they were examined clinically and radiographically to insure normal oral anatomy and rule out any significant oral pathological conditions. All of the animals had mild to moderate

amounts of cervical calculus deposits and exhibited varying degrees of marginal gingivitis. No other pathological conditions were noted.

The teeth selected for inoculation were: all four maxillary central and lateral incisors, the right and left mandibular lateral incisors, and the maxillary and mandibular first premolars on one side of the mouth only (Table I).

In Rhesus monkeys, the nasal aperture extends inferiorly to a considerable distance at the midline, thereby creating a natural partition between the maxillary incisors of the right and left sides (Fig. 26). However, the apices of the central and lateral incisors on each side are so close to one another, that it was decided that lesions forming at these areas would eventually become confluent. For this reason, these teeth were grouped together and their periapical areas treated as one surgical site.

The roots of all four mandibular incisors curve toward each other in these animals, with their apices being very close together (Fig. 27). Since separate contralateral lesions and surgical sites were desired, only the lateral incisors could be operated and still maintain this situation.

The maxillary and mandibular first premolars on one side of the mouth were infected in each animal to serve as sham controls so that unoperated periapical lesions could be observed at different post-inoculation intervals.

After the initial examinations were completed, the monkeys

were anesthetized in the manner already described. Their mouths were held open with a spring-loaded mouth prop. A sterile 556 bur was placed in a high speed handpiece and standard lingual or occlusal endodontic access preparations were made into the pulp chambers of the selected teeth. The antisialogogue effect of the atropine was more than sufficient to prevent salivary contamination of the pulps of the open teeth. A.A.E. standardized #15 root canal files* were sterilized in a glass bead sterilizer and inserted into the root canals. The pulps of the teeth were macerated with the instruments, an effort being made not to withdraw any of the pulpal contents. Pulpal hemorrhage was absorbed by sterile two inch gauze pads held against the teeth.

A sterile 27 gauge long needle was fitted to a disposable 5 cc. syringe and placed into the broth culture of S. faecalis. The broth was drawn up into the syringe and small amounts were then deposited into the pulps of the teeth, with the needle being placed as far apically as it would go into the canals. Afterwards, the endodontic instruments were replaced in the inoculated root canals and further filing was done in an attempt to distribute the organisms as far apically as possible. Cotton pellets moistened with the bacterial culture were placed into the pulp chambers which were then sealed with silver amalgam fillings. Radiographs were taken of the infected teeth after eight weeks in order to observe any periapical changes that might have occurred by that time. Pentobarbital anesthesia was not required to radiograph the animals.

*Star Dental Manufacturing Co., Inc., Conshohocken, Pennsylvania

Phase two

The DABM implant material was prepared according to the method described by Narang and coworkers (137-145).

The long bones of one upper limb (humerus, radius, and ulna) were obtained at necropsy from a Rhesus monkey used in another research project. Immediately upon removal from the animal, the bones were disarticulated and defleshed as completely and aseptically as possible. Employing a reciprocating surgical saw, the bones were cut into cross-sectional pieces approximately 1.0 to 1.5 cm. in length, the epiphyses being discarded. The pieces were then weighed and immersed in a solution of 0.6 N hydrochloric acid previously chilled to 4°C (100 ml. acid per g. of wet bone). The bone was allowed to decalcify in the cold acid for ten days, after which time it was removed, cleaned of all marrow fragments, washed repeatedly in aliquots of sterile physiologic saline and then placed in 70 percent ethanol at 4°C until needed. The time between preparation of the DABM and its use in the animals ranged from nine to eleven months.

At the second operative session, root canal therapy was performed on the infected maxillary central and lateral incisors and mandibular lateral incisors of the animals.

The monkey to be treated that day was placed on the operating table and anesthetized with intravenous pentobarbital sodium in the manner described above. After scrubbing the tooth with an alcohol sponge, a sterile 556 bur in a high speed handpiece was used to remove the amalgam

filling from the access cavity. All pulps were observed to have become necrotic and foul smelling. A #10 or 15 root canal file was placed into the canal and a radiograph was taken to determine the canal length. The root canal was then instrumented and enlarged, an effort being made to stay 1 mm. short of the radiographic apex. The canal was usually enlarged to accommodate a #45 or 50 file. Copious amounts of sodium hypochlorite were used for lubrication and irrigation during canal preparation. Excess irrigant was absorbed on gauze pads and cotton rolls placed around the tooth.

The canal was then prepared for filling by drying with sterile absorbant paper points. Proco-Sol* sealer was mixed and placed into the canal on a sterile reamer. The root canal space was then obliterated by the lateral condensation technique (1) using a standardized gutta-percha master cone** and conventional sized accessory gutta-percha cones***. Excess filling material was removed from the pulp chamber with a heated plugger and the access cavity filled with silver amalgam. Post-filling radiographs were then taken (Fig. 28).

It was originally planned to perform the periapical surgery immediately after root canal therapy had been completed on all the teeth, but the length of time that the animals were kept under anesthesia became a consideration. For their safety, it was decided to postpone the surgery until one or two days later.

*Proco-Sol Nonstaining Root Canal Cement, Proco-Sol Chemical Co., Inc., Philadelphia, Pennsylvania

**Premier Dental Products Co., Norristown, Pennsylvania

***Mynol, Inc., Broomall, Pennsylvania

Prior to surgery, sterile gloves, masks, and gowns were donned, and the animal covered with a sterile drape from the neck down. A standard endodontic surgical armamentarium (Fig. 4) was used for the procedure. After infiltration of the surgical site with lidocaine containing 1:50,000 epinephrine for hemostasis, a Bard-Parker #15 blade mounted on a #2 handle was used to make an incision at the gingival margin, extending from the mesial of the right canine to the mesial of the left canine. Vertical releasing incisions were made at each end of the horizontal incision. Using large surgical curettes and a periosteal elevator, a full thickness mucoperiosteal flap was raised, exposing the underlying cortical plate. Access to the periapical tissues was gained by penetrating the facial cortical plate with a sterile, long shank 558 surgical bur in a high speed handpiece. Physiological saline was used as a coolant to minimize heat damage to the osseous tissues. In some instances, a draining sinus tract (Fig. 5) was present which provided a convenient landmark for surgical penetration. Other times, the position of the apex was estimated as closely as possible using an endodontic file premeasured to the length of the tooth. It was found that some teeth had developed sizeable periapical lesions while others, at least grossly, exhibited none. Those lesions that were discovered were curetted of all soft tissue, otherwise surgical defects of comparable size and location were artificially created.

DABM, which previously had been rinsed in sterile isotonic saline to remove the ethanol, was cut with a scalpel into small pieces

and subsequently implanted into one maxillary and one mandibular defect on the same side. The defects were filled with the implant material as completely as possible, up to the original level of the cortical plate, while the contralateral lesions were allowed to fill with blood clots (Fig. 6). The flaps were then replaced and sutured with either 5-0 Ethiflex* or 4-0 resorbable chromic gut suture material. Post-surgical radiographs were then taken.

To protect against infection, the animals were given intramuscular injections of 2 cc. (600,000 units) of a mixture of benzathine penicillin G and procaine penicillin G** immediately after surgery and then every second day for a total of three doses.

Subsequent to radiographic examination (Fig. 29), the monkeys were killed at four different post-surgical intervals: seven, fourteen, 28, and 90 days. Sacrifice was accomplished painlessly by intravenous injection of 10 cc. of a highly concentrated solution of pentobarbital sodium*** formulated specifically for this purpose. Death occurred within fifteen seconds after administration of the drug. Jaw sections containing the experimental and control teeth were removed with a reciprocating surgical saw and immediately placed in a solution of ten percent neutral buffered formalin. Unnecessary hard tissue was removed with high speed burs to facilitate tissue fixation.

*Ethicon, Inc., Somerville, New Jersey

**Flo-Cillin, Veterinary Products, Bristol Laboratories, Division of Bristol-Myers Co., Syracuse, New York

***Beuthanasia-D Regular, Burns-Biotec Laboratories Division, Chromalloy Pharmaceutical, Inc., Oakland, California

After two weeks, the jaw sections were removed from the fixative and rinsed for 24 hours under running water.

The specimens were decalcified for four to five weeks in a solution of equal parts of 20 percent sodium citrate and 45 percent formic acid. They were subsequently trimmed to eliminate all extraneous tissue and then dehydrated in ethanol, embedded in paraffin and sectioned at six microns. The sections were mounted on glass slides, deparaffinized, hydrated, and stained alternately with hematoxylin and eosin, Masson's trichrome connective tissue stain, and van Gieson's stain for collagen.

The stained histologic sections were examined using a light microscope with magnifications of 40, 100, and 400 diameters. The sections were evaluated according to the quality and degree of inflammation, clot organization, and osseous regeneration observed in the periapical defects.

The radiographic findings were compared to the tissue sections in terms of accuracy of representation of periapical pathology (Tables II-V).

CHAPTER IV

RESULTS

For several days following the inoculation and surgical sessions, the monkeys refused to accept any food. Their eating habits, however, generally returned to normal within three to six days, and they appeared to suffer no lasting ill effects from the anesthesia or the dental procedures.

The clinical, radiographic and histologic findings varied significantly from one animal to another and will be presented separately for each monkey. Since the number and variety of teeth in the Rhesus monkey are identical to that of man, the standard clinical tooth numbering system (#1 to #32) will be utilized to aid in the description of the results.

Monkey 1 - Seven Day Specimens

The animal was a female weighing 4.0 kg. Periapical surgery was performed 128 days after pulpal inoculation. The maxillary left incisors (#9 and #10), and the mandibular left lateral incisor (#23) received DABM implants periapically. Contralateral defects (#7, #8, and #26) were left unfilled. The maxillary and mandibular left first premolars (#12 and #21) were unoperated sham controls.

1. Clinical findings

Prior to surgery, no clinical evidence of periapical pathosis was seen. Upon surgical entry, #23 had a small periapical lesion.

The other periapical defects were entirely due to the surgery. The final sizes of the surgical defects were approximately 6 mm. in diameter in the maxilla and 3 mm. in the mandible.

2. Radiographic findings

Radiographs taken 56 and 128 days after pulpal inoculation were negative for periapical pathology. The defects were visible radiographically after surgery and at the time of death. No difference was noted between implanted and non-implanted defects.

3. Histologic findings

No lesions were seen at the apices of #9 and #10. The implant material had been placed at a distance from the root ends and was separated from them by normal bone. The DABM was easily recognized since it stained less deeply and much more uniformly (with all stains) than the surrounding host bone. The implant was completely engulfed by a blood clot composed of red blood cells and neutrophils in a fibrin lattice (Fig. 8). The lacunae of the DABM were empty, but many of the Haversian canals were filled with the blood clot. At this time period, the implant material appeared to be well tolerated, as no unusual amount of inflammation, beyond that caused by the surgery itself, could be seen. Osteoclastic activity was noted in scattered areas of the host bone surrounding the defect.

Evidence of a chronic periapical lesion was noted around the apex of #23, with a moderate number of lymphocytes and plasma cells observed immediately adjacent to the tooth. The lesion had obviously been

disturbed by the surgery. There was very little evidence of clot formation, merely a relatively small number of scattered red blood cells. There was a considerable amount of host-implant interaction. The DABM border was fuzzy and covered with connective tissue cells in many areas. Macrophages and mesenchymal cells were seen invading the Haversian canals and interfibrillar interstices of the matrix. Many young capillaries were visible as well as the beginning of the formation of excavation chambers in some areas at the border of the matrix. The proliferating cells around the matrix were oriented parallel to its edges, assuming an almost periosteal relationship with it (Fig. 9). No adverse inflammatory response was seen.

Most of the periapical defect around the apices of teeth #7 and #8 was missing from the sections. Apparently, it had been inadvertently trimmed away during preparation.

Tooth #26 had a small, chronically inflamed lesion associated with the lingual surface of the root near the apex. The central portion of the lesion contained lymphocytes, plasma cells and a few scattered neutrophils surrounded by a thin fibrous capsule composed of collagen and fibroblasts. Islands of pale-staining osteoid and new bone spicules were seen beyond the capsule. The root surface adjacent to the lesion contained several small resorptive defects. The surgical lesion was on the opposite side of the root in the facial bone. It did not communicate with the inflammatory lesion. The facial root surface had been gouged with the bur during the surgery. The surgical defect was

filled with a normal appearing blood clot (Fig. 7). Scattered bone and dentin chips were enmeshed in the clot.

Unoperated control lesions were seen at the apices of #12 and #21. The lesions consisted of a central core of chronic inflammatory cells (lymphocytes and plasma cells) surrounded by a fibrous capsule composed of fibroblasts and bundles of collagen (Fig. 24). The lesions associated with both teeth were generally similar except for a greater amount of root resorption at one of the apices of #21.

Monkey 2 - Fourteen Day Specimens

The animal was a female weighing 6.0 kg. The surgical procedure was performed 155 days after bacterial infection of the pulps. The maxillary right incisors (#7 and #8), and the mandibular right lateral incisor (#26) received periapical implants of DABM. The contralateral defects (#9, #10, and #23) were unfilled. Due to the proximity of the apices of #23 and #24, root canal therapy was also performed on the central incisor (#24). The maxillary and mandibular right first premolars (#5 and #28) served as sham controls.

1. Clinical findings

Prior to endodontic treatment, draining sinus tracts were seen associated with all maxillary incisors. The mandibular surgical flap became detached within several days after surgery, and the wound underwent considerable secondary intention healing by the time of sacrifice. During surgery, no periapical lesions could be seen at the apices of #23 and #26. Both maxillary surgical defects measured about 5 mm. in dia-

meter, while the mandibular defects were about 3 mm. across.

2. Radiographic findings

Fifty-five days after pulpal inoculation, small periapical radiolucencies were seen at the apices of all of the upper incisors (#7, #8, #9, and #10) and the maxillary right first premolar (#5). After 155 days, no additional lesions were visible but the ones noted earlier had definitely increased in size. After surgery and at the time of sacrifice, the surgical defects were easily recognizable with no differences between those filled with DABM and those left unfilled.

3. Histologic findings

The defect at the apices of #7 and #8 was fairly extensive and the histologic sections did not contain the root apices. A great deal of host invasion of the DABM was seen at this time period, penetrating all of the Haversian canals and between the matrix fibers. The proliferating host tissue consisted of pale-staining mesenchymal cells, multinucleated clast cells, fibroblasts and abundant capillaries. The boundaries of the implant were indistinct in many areas. Several excavation chambers, filled with host cells, were seen in the matrix. Some new bone was seen attached to the matrix in a few areas where the implant closely approximated the bony margins of the defect (Fig. 13). But, in general, osteoblastic activity was minimal. The tissue was entirely free of inflammation.

In comparison to the maxillary defect, healing in the implanted mandibular defect was far more advanced. The apex of #26 was visible

and was surrounded by a normal periodontal ligament and bone. The DABM had been placed a small distance away from the root apex. As in the maxillary lesion, the matrix exhibited considerable host invasion. However, trabeculae of new bone, coated with osteoblasts and dotted with lacunar osteocytes, had been laid down directly against the DABM in a great many areas (Fig. 12). The new bone was invariably separated from the matrix by a distinct basophilic line. A considerable amount of osteogenetic activity was evident in the sections. The tissue around the matrix and between the spicules of new bone contained many capillaries, fibroblasts, and a relatively loose arrangement of collagen fibers. Inflammation was entirely absent despite the fact that the surgical flap covering the defect had been displaced.

The non-implanted lesion at the apices of teeth #9 and #10 exhibited basically fibrous repair at this time period. Dense collagen, large numbers of spindle-shaped fibroblasts and many young capillaries filled the defect (Fig. 11). A moderate amount of new bone was seen peripherally, emanating from the mature bone at the defect margins. No inflammation was seen.

Healing was less advanced in the non-implanted mandibular defect. A moderate to severe inflammatory infiltrate was seen, containing lymphocytes, plasma cells, scattered neutrophils and a few residual red blood cells (Fig. 10). The lesion appeared very vascular, exhibiting many capillaries. A relatively small number of fibroblasts and collagen fibers were present, especially in the central portion of the

defect. Flap displacement apparently impeded the healing of this lesion a great deal more than the contralateral implanted lesion.

Chronic inflammatory lesions had formed at the apices of the control teeth (#5 and #28). They were similar to each other and contained focal collections of lymphocytes and plasma cells interspersed with capillaries and collagen fibers. They were surrounded by concentric bands of dense collagen fibers and fibroblasts (Fig. 25). Apical tooth structure exhibited resorptive defects, especially #28.

Monkey 3 - Twenty-eight Day Specimens

This animal was a young, well developed male weighing 8.4 kg. Surgery was performed 98 days after pulpal inoculation. The maxillary left incisors (#9 and #10), and the mandibular left lateral incisor (#23) received periapical implants. The contralateral teeth (#7, #8, and #26) were surgerized without implantation of DABM. The maxillary and mandibular left first premolars (#12 and #21) were the sham controls.

1. Clinical findings

The animal developed a draining sinus tract in the apical area of #7 and #8 (Fig. 5). The lesion, upon surgical entry, proved to be quite extensive with a diameter approaching 10 mm. Although no sinus tract had developed on the opposite side (#9 and #10), the lesion was of similar size. Both lower lateral incisors developed periapical lesions which were encountered surgically. The post-surgical mandibular defects were about 3 mm. in diameter (Fig. 6).

2. Radiographic findings

Films exposed 49 days after pulpal inoculation revealed some degree of periapical change around every infected tooth. After 98 days, the lesions all appeared to have grown larger and more radiolucent, especially those associated with the upper incisors. The surgical defects were still visible at the time of sacrifice, but the implanted lesions appeared somewhat smaller and less radiolucent. The differences were not very great, however.

3. Histologic findings

The DABM implanted at the apices of #9 and #10 was invaded extensively by host connective tissue cells. The Haversian canals and interfibrillar spaces were crowded with mesenchymal and osteoprogenitor cells. The implant borders were very indistinct in many areas and blended into the surrounding host tissue. The implant was joined to the host bone at the defect margins and these junctions were always demarcated by basophilic lines. The continuity with host tissue was striking. New bone formation directly against the matrix was evident in areas separated from the defect margins by a considerable distance (Fig. 16). The new bone contained lacunae filled with osteocytes, and its margins were lined by osteoblasts. Excavation chambers filled with multinucleated clastic cells, mesenchymal cells, osteoblasts, and new bone were seen in several areas of the implant. The proliferating connective tissue was very vascular, exhibiting a great many capillaries and some larger vessels. There was no evidence of inflammation in any

of the sections.

The mandibular implant, associated with apex of #23, stimulated a host response that was virtually identical to that seen in the maxillary implant area. It was invaded extensively by host connective tissue, and numerous areas of fusion with host bone, containing basophilic demarcation lines, were seen (Fig. 17, 18). The DABM fibers in some of the implant pieces appeared to be disintegrating.

The apex of #7 obviously was cut off during the surgical procedure. The cut dentin surface was covered with a coating of thin cementum that was continuous with the cementum of the lateral root surface. The lesion was filled with moderately dense fibrous connective tissue composed of collagen fibers and many elongated and angular fibroblasts. Young capillaries were scattered throughout. New bone was seen forming at the periphery of the lesion. In the middle of the healing defect, a small epithelial-lined cavity was seen. The cavity contained red blood cells, many packed degenerating neutrophils, black granular particles suggestive of root canal sealer, and amorphous, non-cellular debris (Fig. 15). This apparent microcyst was surrounded by a fibrous capsule and a mild, chronic inflammatory infiltrate. Inflammation due to trapped particles of root canal filling material, liberated when the apex of #7 was cut, may have stimulated epithelial rests to form a tiny cyst in the center of the defect.

The apex of #26 also was cut during surgery. The cut dentin surface of this tooth also was covered with a layer of cementum, indicating

repair. Adjacent to the apex, black particles of granular root canal sealer were seen associated with a mild, chronic inflammatory infiltrate (Fig. 14). A number of macrophages were seen containing aggregates of phagocytized sealer particles. This small inflammatory area was walled off by a fibrous capsule. The remainder, and by far the major portion, of the lesion exhibited well advanced healing with many small trabeculae of new bone, abundantly covered with osteoblasts. The intertrabecular spaces were filled with young marrow, many blood vessels and some collagen fibers and fibroblasts. No inflammation could be seen except for the area immediately adjacent to the cut root apex.

Both premolar controls (#12 and #21) exhibited chronic periapical lesions with lymphocytes and plasma cells surrounded by fibrous capsules. Root resorption was seen at different points along the perimeter of the apices adjacent to the lesions.

Monkey 4 - Ninety Day Specimens

This animal was a female weighing 5.3 kg. Surgery was performed 80 days after inoculation of the pulps. The maxillary right incisors (#7 and #8), and the mandibular right lateral incisor (#26) received DABM implants in their periapical areas, while the corresponding contralateral areas (#9, #10, and #23) were allowed to heal by clot organization. The maxillary and mandibular right first premolars (#5 and #28) were sham controls.

1. Clinical findings

At the time of surgery, no clinical indication of periapical pathosis was noted in any area. At operation, small periapical lesions were encountered at the apices of all maxillary incisors (#7, #8, #9, and #10) and the mandibular left lateral incisor (#23). The maxillary surgical defects were 5 mm. in diameter, while those in the mandible measured about 3 mm.

2. Radiographic findings

At both 56 and 80 days after pulpal inoculation, teeth #7, #8, #10 and #23 demonstrated periapical rarefactive areas. The lesions appeared somewhat larger at the latter observation period. Immediately after surgery, all the defects clearly were evident radiographically. At sacrifice, both mandibular defects appeared completely healed. The maxillary implant site appeared more radioopaque than the surrounding bone while the non-implanted site still appeared somewhat radiolucent.

3. Histologic findings

The maxillary implant site (#7 and #8) displayed complete regeneration. One of the apices obviously had been cut off during the surgery and a large piece of osteocementum, apparently containing DABM fragments, appeared fused to the tooth apex (Fig. 21). The roots of both teeth were surrounded entirely by normal periodontal ligament. Fragments of DABM were seen, totally incorporated into the normal bony trabeculae of the healed defect (Fig. 22). The DABM was distinguished easily, as it appeared more homogeneous and stained a slightly different

color than the surrounding vital bone, and usually displayed a basophilic demarcation line around its perimeter. Since the defect originally had been filled with the implant material, and only small incorporated fragments remained after 90 days, the matrix must obviously have undergone resorption and replacement by the host tissue. An interesting observation was that the lacunae within the remaining matrix appeared to have been repopulated with cells (Fig. 23). The intertrabecular spaces contained normal fatty marrow and mature blood vessels. The tissue was without any trace of inflammation.

The mandibular implant site (#26) had also regenerated completely and looked exactly like the maxillary defect just described. The apex of #26 was blunted and covered with a thin layer of cementum indicating that it had also been cut during surgery.

The histologic sections of the maxillary non-implanted defect (#9, and #10) looked very different. A large chronic lesion was present, exhibiting a huge concentration of lymphocytes and plasma cells interspersed with collagen bundles, fibroblasts and small blood vessels (Fig. 19). Resorptive lacunae, containing osteoclasts, were seen in the surrounding host bone. There was no evidence of new bone formation in any area of the lesion.

The mandibular non-implanted defect periapical to #23 had undergone almost complete regeneration except for the area immediately adjacent to the apex. A small chronic lesion was seen, containing lymphocytes, plasma cells and many small vessels. Especially interesting was

the observation of epithelial proliferation within this residual lesion. Arcading strands of epithelial cells were seen, haphazardly arranged within the substance of the inflammatory infiltrate (Fig. 20). The lesion was encased in a moderately dense fibrous capsule.

The maxillary right first premolar (#5) exhibited a chronic periapical lesion at one of its apices that appeared very similar to that seen with other control teeth in the other three monkeys. The apices of the mandibular control tooth (#28) were not visible in the sections and could not be evaluated.

CHAPTER V

DISCUSSION

The use of Streptococcus faecalis

In a study to elucidate the nature of the bacterial flora of root canals, Leavitt, et al (147) indicated, in 1958, that the streptococci were the largest single group of organisms present.

In 1961, Engstrom and Frostell (148), and Crawford and Shankle (149) reported similar studies and drew the same conclusions. Additionally, in the latter study, the investigators noted that on the initial culture of a tooth that had been left open to the oral environment, enterobacteria were among the most common organisms retrieved.

Torneck (150) described a study performed at the University of Toronto Dental School in which a high proportion of staphylococci and enterococci had been isolated from acutely infected dental pulps. Particularly interesting was the presence of enterococci in cultures taken from teeth with a history of previous endodontic treatment.

The Streptococcus faecalis organism, in particular, has been implicated as a significant pulpal pathogen. Engstrom (151) studied the significance of enterococci in root canal therapy. He found that in 65 percent of teeth infected with enterococci, S. faecalis being the usual isolate, the first antiseptic treatment failed to rid the canal of the organism. This was in comparison to the figure of 39.5 percent for teeth infected with other varieties of bacteria. He concluded that

enterococcal infections of the pulp cavity constituted a definite treatment problem because they were difficult to eliminate and caused the period of treatment to be prolonged.

In a study reporting the results from over 4,000 root canal cultures, Winkler and van Amerongen (152) noted that S. faecalis was the most common isolate to occur in pure culture. This organism was found to be present more frequently in subsequent cultures than initial cultures from teeth with necrotic pulps, and to persist tenaciously in root canals once established. With regard to S. faecalis and its variant, S. liquefaciens, the authors concluded that these organisms seemed to be able to cause clinical infections that were very difficult to eliminate and therefore should be considered potentially pathogenic for the dental pulp.

Mazukelli (153), using a labelled (streptomycin resistant) strain of S. faecalis, was able to reliably induce periapical pathosis in dogs within four months after pulpal inoculation with the organism.

After considering the evidence presented above, it was decided that S. faecalis was the organism of choice for infecting the pulps of the monkeys used in this study. The chosen organism did, in fact, prove to be quite effective in this regard, as only five out of 23, or 21.7 percent, of the periapical areas examined failed to display either clinical, microscopic or radiographic evidence of pathologic change. Since all of the teeth that were treated endodontically were found to exhibit

at least pulpal necrosis, it may be asserted that given enough time to develop, periapical pathosis would have been found in all of the areas.

A further consideration in this regard is the fact that periapical lesions of bacterial etiology are the result of three interrelated factors: the number of invading organisms, the virulence of the organism, and the intrinsic ability of the host to resist infection. This triad of factors was discussed by Zeldow and Ingle (154), who stated that their relationship could be expressed in the following equation:

$$\frac{\text{Number} \times \text{Virulence}}{\text{Resistance}} = \text{Severity of disease state}$$

Insofar as this relates to the current experiment, the virulence and resistance factors could not be measured or controlled. Virulence is inherently an in vivo quality of an organism and may vary from one anatomic site to another in the host. Aside from maintaining their normal state of health, there was no way to determine each monkey's individual ability to resist periapical infection. In contrast, however, the number of infecting organisms was a controllable factor. Although relatively comparable volumes of the broth culture were used to infect the various teeth, no attempt was made to standardize the inoculum since it did not appear crucial to the outcome of the project. Perhaps this was an error.

Periapical defect variations

It certainly would have been preferable for all of the experimental defects, both implanted and non-implanted, to have resulted from

periapical pathosis, as this was the intended result of the first phase of the experiment. Artificially created bony lesions are not really comparable to lesions resulting from chronic disease and the different environments may have affected the results of the DABM implants. This must be borne in mind in the evaluation of the results of this research effort.

Within the time constraints allotted for completion of the project, it was desired that the pathologic lesions be as large as possible. Consequently, four different time intervals transpired between the pulpal inoculation and surgical procedures, the longer post-inoculation intervals being coupled with the shorter post-surgical sacrifice times. Admittedly, this added another variable to the experiment and, on that basis, may be cause for criticism.

A further criticism that may be leveled is that the periapical defects were not all of the same dimensions. Generally, the mandibular lesions, which were smaller than the maxillary lesions, appeared to exhibit more advanced healing at any given post-surgical time period. In point of fact, however, periapical surgery on the anterior teeth of Rhesus monkeys is fraught with more than its share of difficulties. The apices of the maxillary incisors are separated from the nasal cavity by a thin cortical plate of bone, the distance being only one or two millimeters. Great care had to be exercised to prevent the creation of an oral-nasal defect which would certainly have adversely affected healing in these areas. Surgical caution consequently resulted in the defects

being somewhat lateral to the root apices in the cases where no periapical lesions were present to serve as landmarks.

A similar problem was created by the proximity of the apices of the mandibular central and lateral incisors. In the fourteen day animal, the apices of teeth #23 and #24 actually appeared to overlap, and there was no way to avoid devitalizing #24 during the surgery. For this reason, endodontics was performed on the latter tooth even though its pulp had not been infected. In general, surgical entry was begun in the area distal to the lateral incisor apex to avoid the central. This resulted in nicking the canine roots in a few instances, implantation of the DABM at a slight distance from the root apex, and, in one case, failure to note and curette a true pathologic lesion. Although the surgical defects were not all of the same size, contralateral defects within the same animal were all of comparable dimensions.

Other problems associated with the use of Rhesus monkeys include the high initial cost of the animals, their propensity to contract communicable diseases while in captivity, and their relative physiological fragility with respect to surgical anesthesia. Nevertheless, their anatomic and phylogenetic similarity to man make them highly desirable as experimental models in clinically oriented research projects.

Radiographic findings

Bhaskar and Rappaport (155) created periapical lesions in dogs by exposing the pulps and leaving them open to the oral environment for nine months. Although all of the teeth developed periapical lesions his-

tologically, radiographic findings were negative in many of the areas, especially with respect to the anterior teeth, where radiographic and microscopic findings were concordant in only 17.6 percent of the cases.

In the present study, the radiographic and histologic or clinical findings were in agreement in seventeen of 23 or 73.9 percent of the areas, prior to surgical intervention. In contrast to the results of Bhaskar and Rappaport, the anterior films proved to be correct more often than the posterior films, with 87.5 percent proving to be accurate.

All of the lesions that failed to present radiographically, proved to be quite small when viewed clinically or microscopically. Because of their small size, it is very unlikely that any of them involved resorption of the inner surfaces of the adjacent osseous cortical plates. According to Bender and Seltzer (156), and Schwartz and Foster (157), periapical lesions are detectable radiographically only if there is a perforation of the bony cortex, erosion from the inner surface of the cortex, or extensive erosion or destruction from the outer cortex.

Effect of DABM implants

The biocompatibility of the DABM implants was uniformly excellent. There was not the slightest clinical or histologic evidence of host rejection of the material in any area at any post-surgical interval. This finding is in agreement with the results noted by Narang and coworkers (137-145).

As early as seven days after surgery, the mandibular DABM im-

plant appeared to stimulate considerable proliferative activity on the part of the host bed. Haversian canals and interfibrillar spaces in the bone matrix were invaded by host connective tissue cells, and the implant-host interface was seen to be extremely cellular, having an almost periosteal appearance. This early host response is in agreement with the findings of Huggins and Urist (131). Although they used decalcified allogeneic dentin, they felt that the cytochemical influences of bone and dentin matrix on host tissue were probably comparable.

In contrast to the mandibular implant, the maxillary implant, at seven days, was engulfed in a blood clot and appeared relatively inert. This difference was probably due to the size of the maxillary defect, which provided space for a large clot to form, requiring more time for organization and denying host connective tissue cells access to the inductive substrate.

After fourteen days, host bone was seen in direct physical apposition to the DABM. The phenomenon was especially notable in the mandibular defect. Wherever the new bone contacted the implant, a distinct basophilic line could be seen. These demarcation lines made new bone easy to distinguish from the matrix and were described by Urist (118), Yeomans and Urist (123), and Bang and Urist (125).

The advanced healing demonstrated in the mandibular implant site is especially interesting in view of the fact that the surgical flap was displaced and the area subject to irritation and contamination. The contralateral defect, which had been treated exactly the same way during

the surgical procedure, except for insertion of DABM, was chronically inflamed and demonstrated very little repair. Perhaps the implant material served as a physical barrier, limiting the ability of environmental contaminants to enter the bone lesion and disturb the healing process.

The formation of excavation chambers in DABM implants is a well established phenomenon and has been cited by Urist and his co-workers in numerous publications (118, 120, 123, 125, 131). The beginning of the formation of excavation chambers was noted after seven days. One would assume that these cell-filled depressions in the matrix are formed through the action of multinucleated clastic cells, but relatively few of these cells could be visualized in association with the excavation chambers at any of the time periods.

At fourteen days, the non-implanted maxillary defect demonstrated considerable organization. The lesion was filled with dense fibrous connective tissue and exhibited some new bone at its periphery. New bone formation had apparently progressed as well in this defect as it had in the contralateral implanted defect, so that neither one could be considered superior at this time period.

At 28 days, many more points of union were visible between the new host bone and the DABM implants. Connective tissue invasion was far more advanced. In some areas it actually was difficult to make a determination of exactly where host fibrous tissue ended and DABM began.

The non-implanted defects were healing quite well by this time.

The mandibular was more advanced, in terms of bone formation, than the maxillary. Apparently, some root canal filling material had been released into both defects during the surgery. The tissue responses were different, however, in the two areas. The maxillary response was epithelial proliferation with resultant cyst formation. The presence of an epithelial-lined cystic cavity was unquestionable. The mandibular defect displayed a foreign body response with lymphocytes, plasma cells, macrophages and a fibrous capsule.

The implanted defects did not appear to contain any more bone than the non-implanted defects and still could not be considered to exhibit an advanced state of repair.

After 90 days, the implanted defects had completely regenerated, displaying mature bone, marrow, and periodontal ligament. Quite interesting was the finding of fragments of DABM totally encased in bony trabeculae. Apparently, the bulk of the DABM had been resorbed and replaced by new host bone, and these fragments either were still undergoing resorption or were inert and inaccessible to further change. More interesting still, was the finding of repopulation of the lacunae within these fragments. It is highly unlikely that the cells were osteocytes, since osteocytes are incorporated only as bone is laid down, and the DABM fragments were not produced by the host. It is more likely that they were some other type of connective tissue cell.

The non-implanted defect in the mandible appeared to be almost completely filled with small bone trabeculae. The area immedi-

ately adjacent to the root apex was chronically inflamed and showed distinct epithelial proliferation in the form of arcading cells, as originally described by Shear (18). This chronic proliferative reaction may have been due to residual periapical filling material, such as sealer particles, that was not visible in any of the particular sections examined.

The histologic sections of the 90 day maxillary non-implanted defect were by far the most surprising. A large chronic lesion, apparently a granuloma, filled the entire defect. No new bone was visible. In fact, host bone was still actively being resorbed. Surgically, the area had been treated in exactly the same manner as the contralateral defect, except for the fact that no DABM had been placed in it. It is very unlikely that just one side could have been surgically contaminated.

The implanted sites definitely looked better at 90 days, since they had completely healed and exhibited no trace of inflammatory activity. There was a tremendous difference between the 28 day and the 90 day specimens and it would have been interesting to have had an additional specimen displaying the state of healing after 60 days.

Considering the small number of animals and extremely limited number of post-surgical specimens, it is difficult to make any definite statements regarding the osteogenic potential of the DABM implant material from the results obtained in this study alone. Healing was improved at the implant sites in the 90 day animal but not significantly

in the other three monkeys.

It also cannot be stated with certainty that the DABM induced new bone to form by the process of autoinduction as described by Bang and Urist (125). Bone did indeed form in direct apposition to the DABM, but it did so in an environment where bone should normally be formed as part of the healing process.

What was significant, however, was the finding at 28 days of new bone directly against the implant in an area of the lesion considerably removed from its bony margin. Osteogenetic activity normally occurs at the margins of a defect, progressing towards its center and slowly decreasing its size. It doesn't usually occur spontaneously in the interior of a defect. It is definitely possible that resorption of the implant material by host connective tissue cells resulted in the liberation of a bone induction principle which stimulated competent cells to differentiate into osteoblasts and lay down new bone in this relatively unusual central location.

Urist and his coworkers (117-132) have certainly established that bone formation by autoinduction does occur in the presence of properly prepared inductive substrates, and there is no good reason why it could not have taken place in this study.

Despite the fact that bone induction was not unequivocally proven to have occurred in this study, the excellent host tolerance and vigorous connective tissue response to the DABM was most encouraging and definitely warrants continued investigation of the unique potentialities of this material.

CHAPTER VI

CONCLUSIONS

1. Streptococcus faecalis is capable of producing periapical pathologic lesions in Rhesus monkeys.
2. Decalcified allogeneic bone matrix that has been stored in 70 percent ethanol for nine to eleven months, is extremely well tolerated by the periapical tissues of Rhesus monkeys.
3. Decalcified allogeneic bone matrix stimulates proliferation of host connective tissue, and is actively invaded by this tissue within seven days of implantation into periapical defects in Rhesus monkeys.
4. New host bone forms in direct physical apposition to decalcified allogeneic bone matrix within fourteen days of its implantation into periapical defects in Rhesus monkeys.
5. Periapical surgery of the anterior teeth in Rhesus monkeys is very difficult due to anatomic constraints.
6. Further research with decalcified allogeneic bone matrix on the healing of bony defects is indicated.

CHAPTER VII

SUMMARY

Selected teeth in four adult Rhesus monkeys were inoculated with a pure culture of Streptococcus faecalis in order to create periapical lesions in the jaws of the animals. After eleven to 22 weeks, most of the inoculated teeth were treated endodontically. The rest served as sham controls. Subsequent to the root canal procedures, periapical surgery was performed. Half of the surgical sites received implants of decalcified allogeneic bone matrix, while the remaining ones were allowed to fill with a blood clot. The animals were sacrificed after seven, fourteen, 28, and 90 days. The jaws were removed at necropsy and after fixation, decalcification, and histologic preparation, the surgical sites were evaluated microscopically.

At the seven, fourteen, and 28 day post-operative intervals, neither the implanted nor the non-implanted areas exhibited superior healing. After 90 days, the implanted sites had completely regenerated, while the non-implanted sites exhibited residual areas of chronic inflammation.

The decalcified allogeneic bone matrix was very well tolerated in all of the animals, and stimulated host connective tissue to proliferate and invade the implants as early as seven days post-operatively. By fourteen days, bone was seen in direct apposition to the implant material.

The small number of animals precluded any definitive conclusions regarding the osteogenic properties of decalcified allogeneic bone matrix implants.

CHAPTER VIII

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CHAPTER IX

APPENDIX

A. Tables

B. Figures

TABLE I

EXPERIMENTAL DESIGN

Monkey	Interval from inoculation to surgery	Post- surgical sacrifice interval	<u>Maxillary Lesions</u>		<u>Mandibular Lesions</u>		Non-surgical inoculated sham controls
			DABM implant	No implant	DABM implant	No implant	
1	128 days	7 days	#9,10	#7,8	#23	#26	#12,21
2	155 days	14 days	#7,8	#9,10	#26	#23	#5,28
3	98 days	28 days	#9,10	#7,8	#23	#26	#12,21
4	80 days	90 days	#7,8	#9,10	#26	#23	#5,28

TABLE II

7 DAY SPECIMENS

Site	Pathologic lesion?	Clinical signs	Radiographic findings		Surgical defect size (mm)	Treatment	Histologic findings
			56 days	128 days			
#7,8	No	None	Neg	Neg	6	No implant	Section overtrimmed
#9,10	No	None	Neg	Neg	6	DABM implant	Blood clot around implant and in Haversian canals
#26	Yes	None	Neg	Neg	3	No implant	Blood clot
#23	Yes	None	Neg	Neg	3	DABM implant	Moderate connective tissue invasion of implant; beginning of excavation chambers
#12	Yes	None	Neg	Neg		Control	Chronic lesion
#21	Yes	None	Neg	Neg		Control	Chronic lesion

TABLE III

14 DAY SPECIMENS

Site	Pathologic lesion?	Clinical signs	Radiographic findings		Surgical defect size (mm)	Treatment	Histologic findings
			55 days	155 days			
#7,8	Yes	Draining sinus	Pos	Pos (larger)	5	DABM implant	Much connective tissue invasion; excavation chambers; areas of fusion with host bone near defect margins
#9,10	Yes	Draining sinus	Pos	Pos (larger)	5	No implant	Fibrous repair; some new bone at lesion periphery
#26	No	None	Neg	Neg	3	DABM implant	Same as #7,8 but more areas of fusion with host bone; basophilic demarcation lines; much osteoblastic activity *
#23	No	None	Neg	Neg	3	No implant	Chronic inflammation; little fibrous repair *
#5	Yes	None	Pos	Pos (larger)		Control	Chronic lesion
#28	Yes	None	Neg	Neg		Control	Chronic lesion

* Mandibular surgical flap was displaced

TABLE IV

28 DAY SPECIMENS

Site	Pathologic lesion?	Clinical signs	Radiographic findings 49 days 98 days		Surgical defect size (mm)	Treatment	Histologic findings
#7,8	Yes	Draining sinus	Pos	Pos (larger)	10	No implant	Advanced fibrous repair; microcyst; much new bone at lesion periphery
#9,10	Yes	None	Pos	Pos (larger)	10	DABM implant	Striking continuity with host tissue; bone formed against implant at interior of defect
#26	Yes	None	Pos	Pos (larger)	3	No implant	Chronic inflammation seen around sealer particles; phagocytized sealer; advanced healing; much new bone at interior of lesion
#23	Yes	None	Pos	Pos (larger)	3	DABM implant	Same as #9,10 but more new bone against implant
#12	Yes	None	Pos	Pos (larger)		Control	Chronic lesion
#21	Yes	None	Pos	Pos (larger)		Control	Chronic lesion

TABLE V

90 DAY SPECIMENS

Site	Pathologic lesion?	Clinical signs	Radiographic findings 56 days 80 days		Surgical defect size (mm)	Treatment	Histologic findings
#7,8	Yes	None	Pos	Pos (larger)	5	DABM implant	Complete regeneration; DABM fragments incorporated into host bone; repopulated implant lacunae
#9,10	Yes	None	Pos	Pos (larger)	5	No implant	Large granuloma; active resorption at osseous margins
#26	No	None	Neg	Neg	3	DABM implant	Complete regeneration; same as #7,8
#23	Yes	None	Pos	Pos (larger)	3	No implant	Almost complete bone fill; small residual apical lesion with epithelial proliferation
#5	Yes	None	Neg	Neg		Control	Chronic lesion
#28	?	None	Neg	Neg		Control	Root apices not visible in histologic sections

Figure 1: Large periapical lesion, approximately 2 cm. in diameter, associated with the canine and first premolar. Communication with the maxillary sinus was noted during surgical enucleation. Lesion was a periapical cyst. Microscopic diagnosis courtesy of Dr. Patrick Toto. (Magnification, X2.5.)

Figure 2: Periapical radiolucency creating a diagnostic dilemma. Patient had no clinical symptoms and stated that periapical surgery had been performed on the tooth several years previously. A provisional diagnosis of periapical scar was made and the patient placed on three month recall. (Magnification, X2.5.)



Figure 3: Method of radiographing the Rhesus monkeys. Note hand-held laboratory x-ray unit and spring-loaded mouth prop. Operator wore lead-lined gloves and a lead-lined apron.



Figure 4: Standard surgical armamentarium used for periapical surgery on the animals.



Figure 5: Draining sinus tract perforating the facial cortical plate. Photograph taken prior to osseous penetration. Monkey 3, area #7,8.



Figure 6: Mandibular surgical sites on monkey 3. Photograph taken prior to flap replacement. Note DABM implant (arrow).



Figure 7: Seven day specimen (monkey 1) of mandibular non-implanted defect. Note blood clot (C); cut surface of cancellous bone (Bo); artifact space (A). (Hematoxylin and eosin stain. Original magnification, X25.)



Figure 8: Seven day specimen (monkey 1) of maxillary implanted defect. Note DABM implant (Imp) surrounded by blood clot (C). Ha-versionian canals in matrix are filled with blood clot (arrows). (Hematoxylin and eosin stain. Original magnification, X40.)

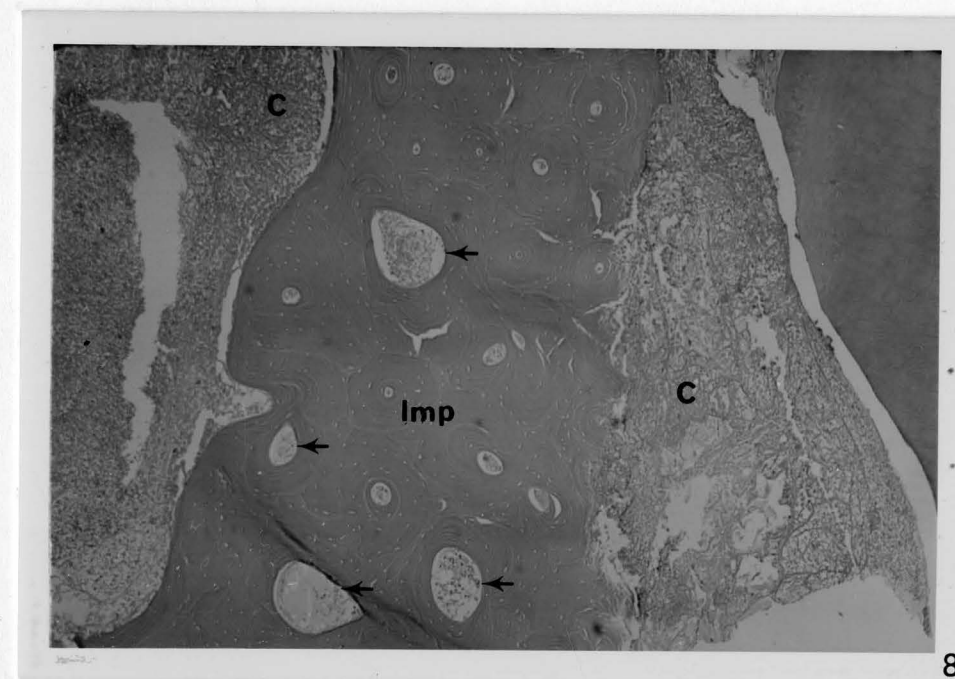
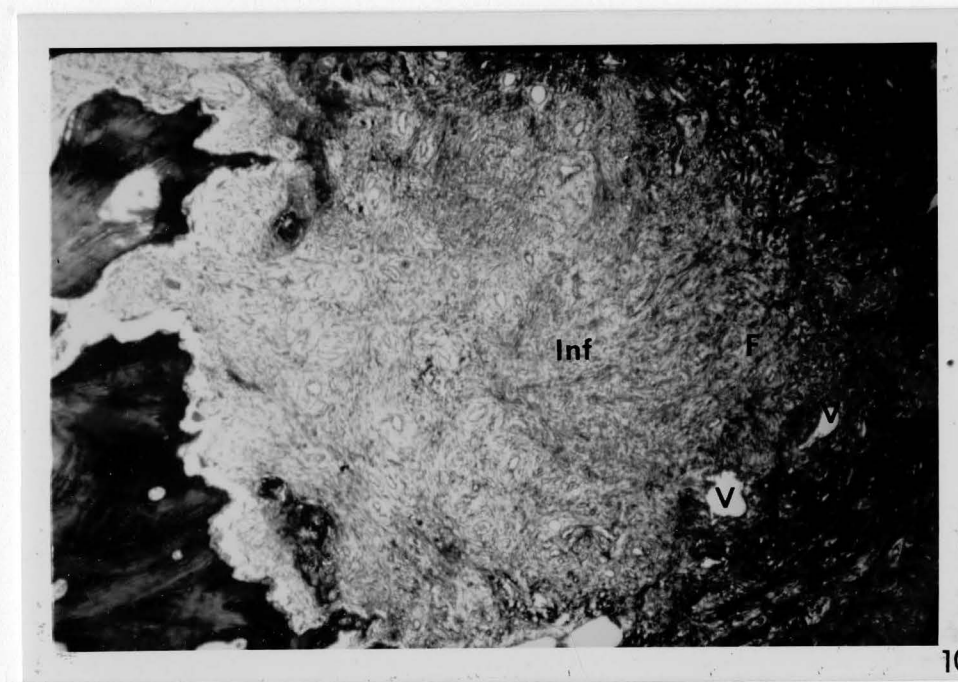


Figure 9: Seven day specimen (monkey 1) of mandibular implanted defect. Note considerable proliferation of host connective tissue (arrows) around perimeter of bone matrix (M). Almost complete absence of inflammation. (Masson's trichrome connective tissue stain. Original magnification, X40.)



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Figure 10: Fourteen day specimen (monkey 2) of mandibular non-implanted defect. Note considerable inflammation (Inf) at center of lesion, with fibrous tissue (F) and blood vessels (V) at periphery. (Masson's trichrome connective tissue stain. Original magnification, X25.)



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Figure 11: Fourteen day specimen (monkey 2) of maxillary non-implanted defect. Note highly vascular fibrous connective tissue filling the defect, with several large vessels (V) and some peripheral formation of new bone (arrows). (Masson's trichrome connective tissue stain. Original magnification, X25.)



Figure 12: Fourteen day specimen (monkey 2) of mandibular implanted defect. Note trabeculae of new host bone (NB) in direct physical apposition (arrows) to DABM implant material (Imp). Complete lack of inflammation. (Hematoxylin and eosin stain. Original magnification, X25.)

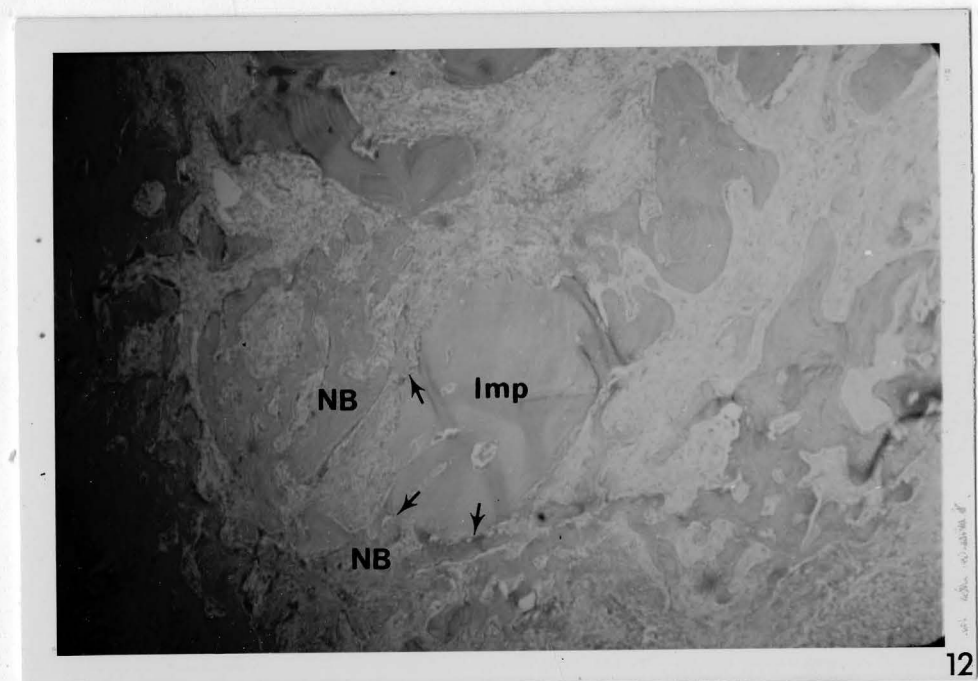
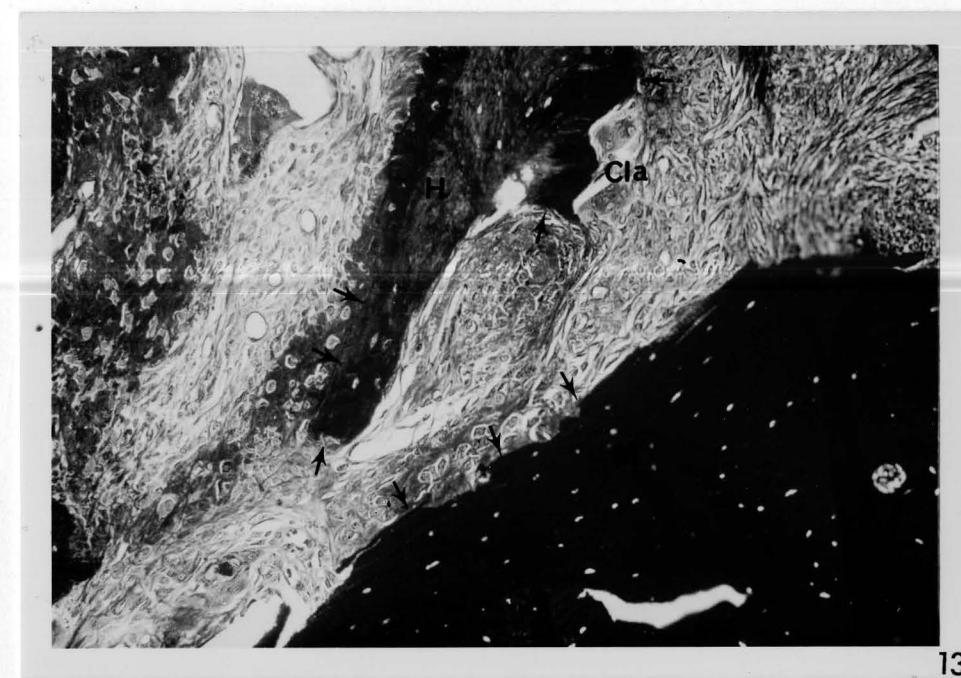
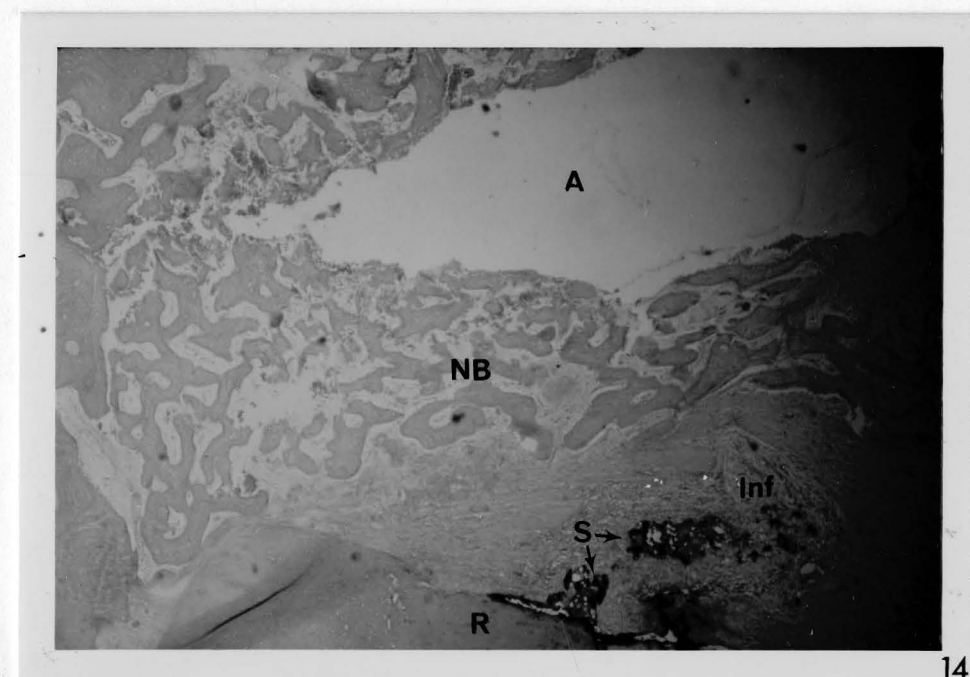


Figure 13: Fourteen day specimen (monkey 2) of maxillary implanted defect. Note new bone (arrows) forming against mature host bone (H) and bone matrix implant (M). Osteoclasts (Cla) in Howship's lacunae are resorbing host bone. (Masson's trichrome connective tissue stain. Original magnification, X100.)



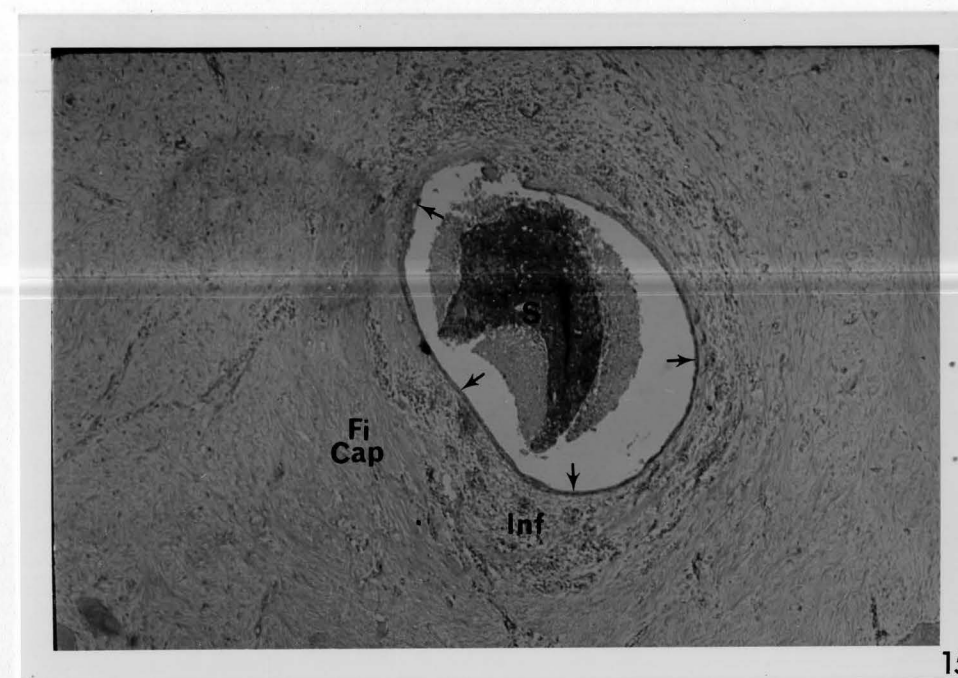
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Figure 14: Twenty-eight day specimen (monkey 3) of mandibular non-implanted defect. Note cut root surface (R), scattered particles of root canal sealer (S), and associated chronic inflammatory response (Inf). A considerable amount of new bone can be seen adjacent to the artifact space (A). (Hematoxylin and eosin stain. Original magnification, X25.)



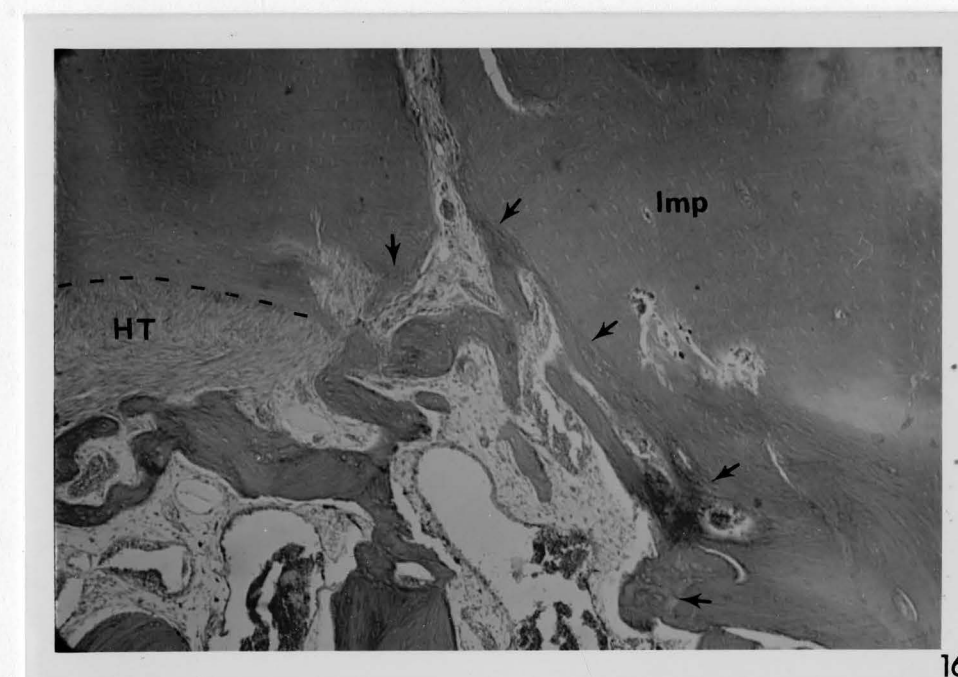
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Figure 15: Twenty-eight day specimen (monkey 3) of maxillary non-implanted defect. Lesion is completely filled with dense, fibrous connective tissue. In center of lesion is a microcyst lined by epithelium (arrows), containing scattered particles of root canal sealer (S) associated with packed degenerating red blood cells and neutrophils. The microcyst is surrounded by a moderate inflammatory infiltrate (Inf) and a fibrous capsule (Fi Cap). (Hematoxylin and eosin stain. Original magnification, X40.)



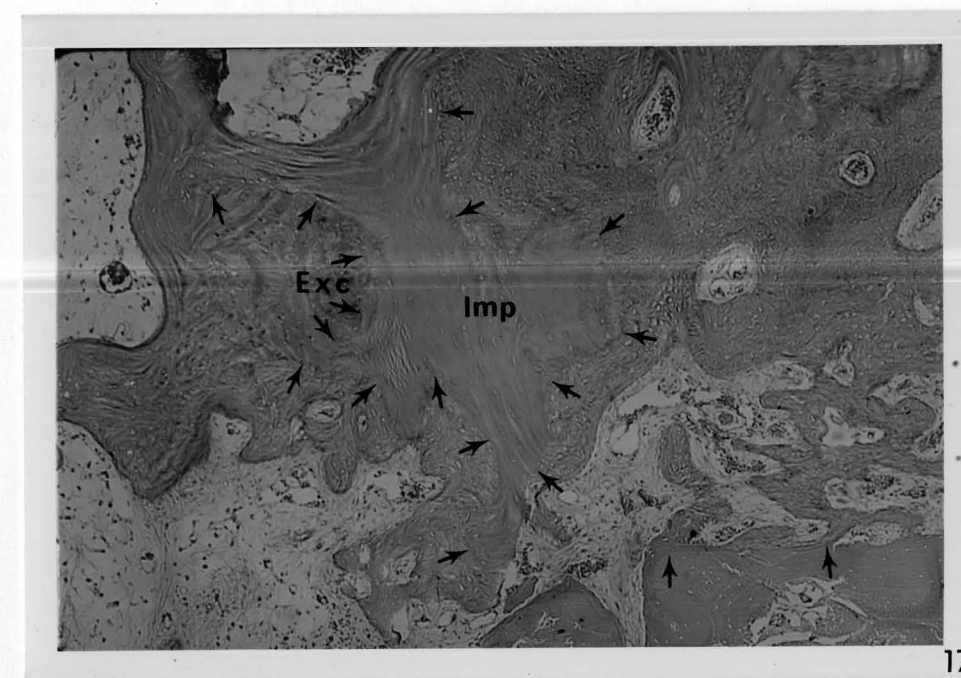
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Figure 16: Twenty-eight day specimen (monkey 3) of maxillary implanted defect showing new host bone (arrows) in direct apposition to the DABM implant (Imp). Border between host connective tissue (HT) and implant is indistinct (dashes). (Hematoxylin and eosin stain. Original magnification, X40.)



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Figure 17: Twenty-eight day specimen (monkey 3) of implanted mandibular defect showing advanced incorporation of DABM (Imp) into new host bone. Arrows indicate implant borders which are indistinct. An excavation chamber (Exc) filled with new bone can be seen in the bone matrix implant. (Hematoxylin and eosin stain. Original magnification, X40.)



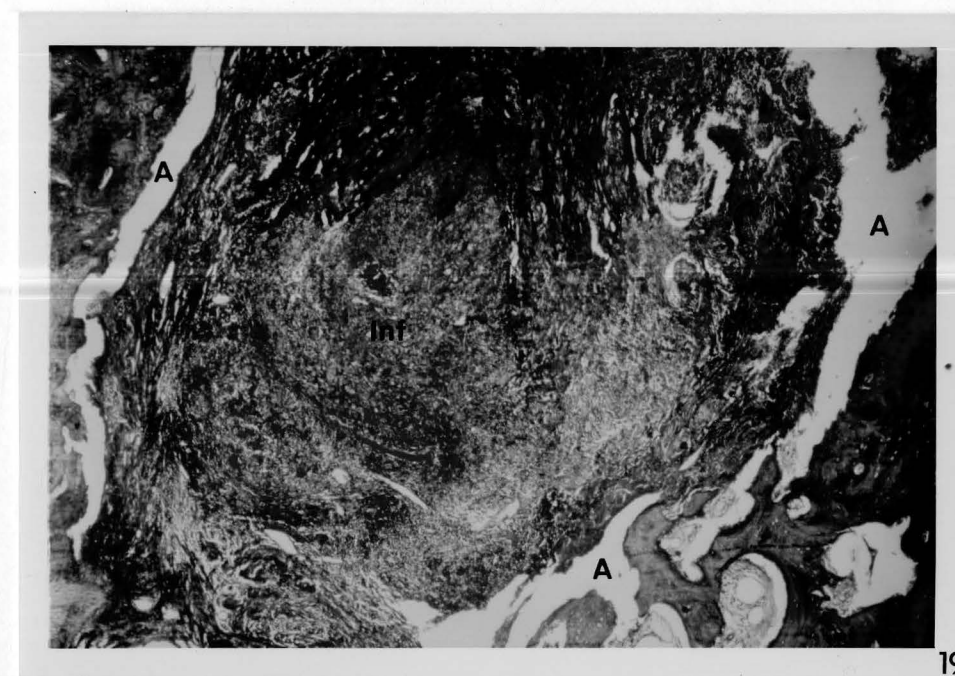
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Figure 18: Twenty-eight day specimen (monkey 3) of implanted mandibular defect. Higher magnification of same specimen seen in Fig. 17. Border between new bone and implant (Imp) is quite indistinct (arrows), suggesting an inductive process. (van Gieson's collagen stain. Original magnification, X100.)



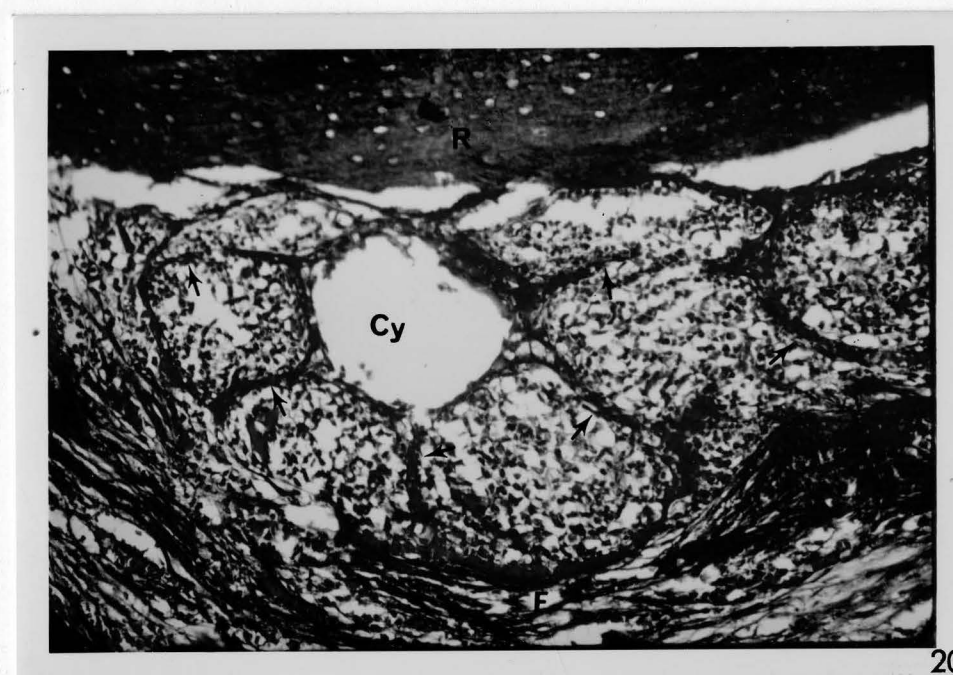
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Figure 19: Ninety day specimen (monkey 4) of non-implanted maxillary lesion. Note considerable inflammatory activity (Inf) surrounded by dense, fibrous tissue. Artifact spaces (A) separate lesion from surrounding bone. (Masson's trichrome connective tissue stain. Original magnification, X25.)



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Figure 20: Ninety day specimen (monkey 4) of non-implanted mandibular defect. Note root end (R), proliferating strands of epithelium (arrows), small cystic cavity (Cy), and dense collagen fiber bundles (F). (Masson's trichrome connective tissue stain. Original magnification, X100.)



20

Figure 21: Ninety day specimen (monkey 4) of implanted maxillary defect showing root apex (R), normal periodontal ligament (Pdl), and artifact space (A). A piece of osteocementum has fused with the root apex and contains scattered fragments of DABM implant material (arrows). (Hematoxylin and eosin stain. Original magnification, X40.)

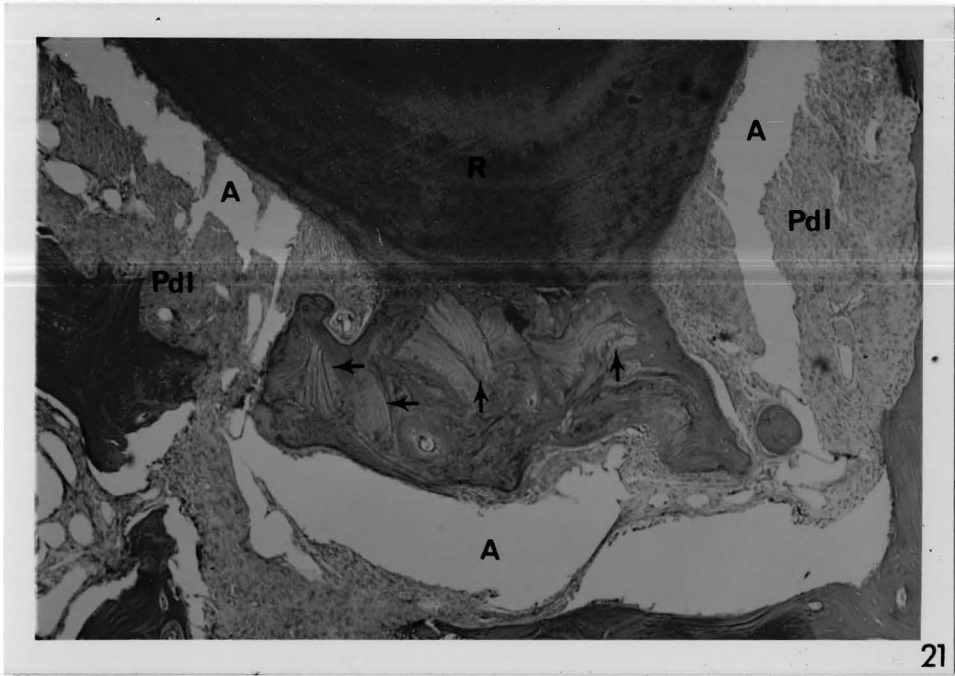


Figure 22: Ninety day specimen (monkey 4) of implanted maxillary defect showing area apical to that seen in Fig. 21. Note completely normal histologic appearance of tissue, including fatty marrow (Mar) and mature bone trabeculae containing scattered fragments of DABM (arrows). (Hematoxylin and eosin stain. Original magnification, X40.)

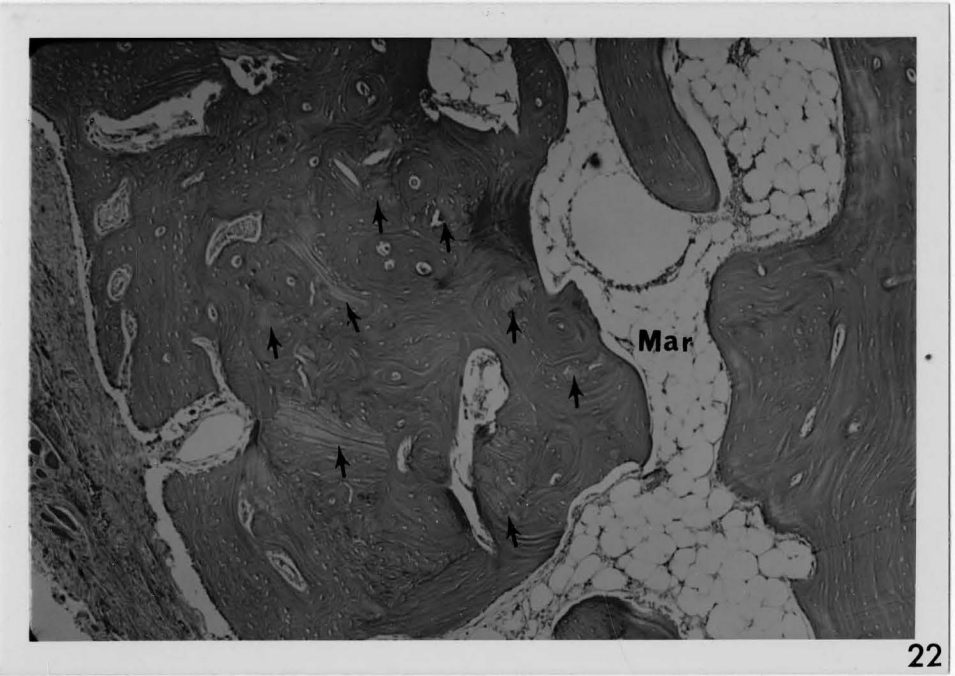
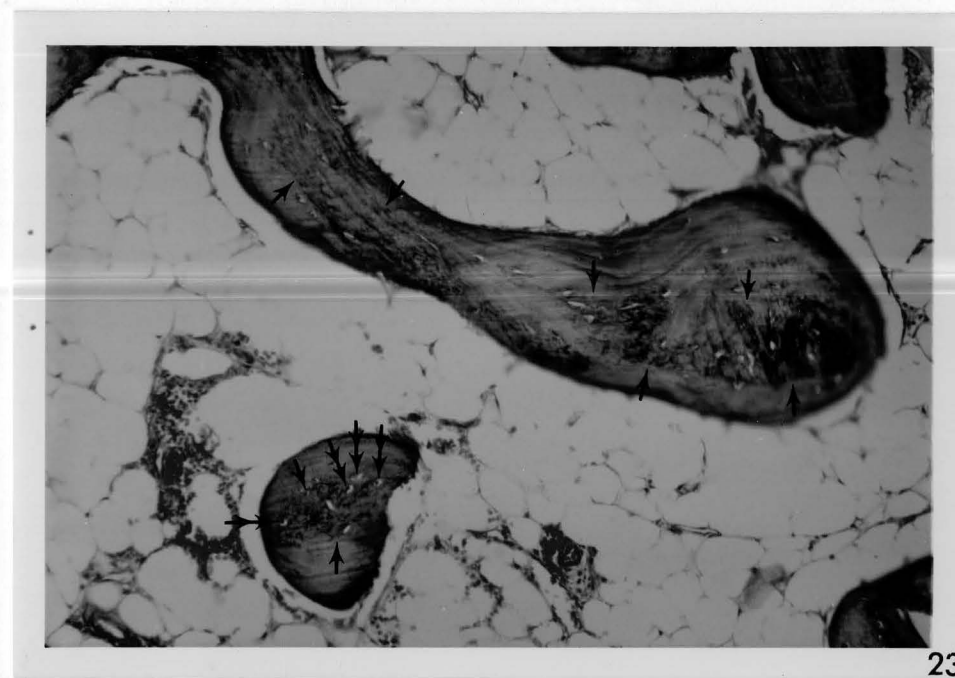
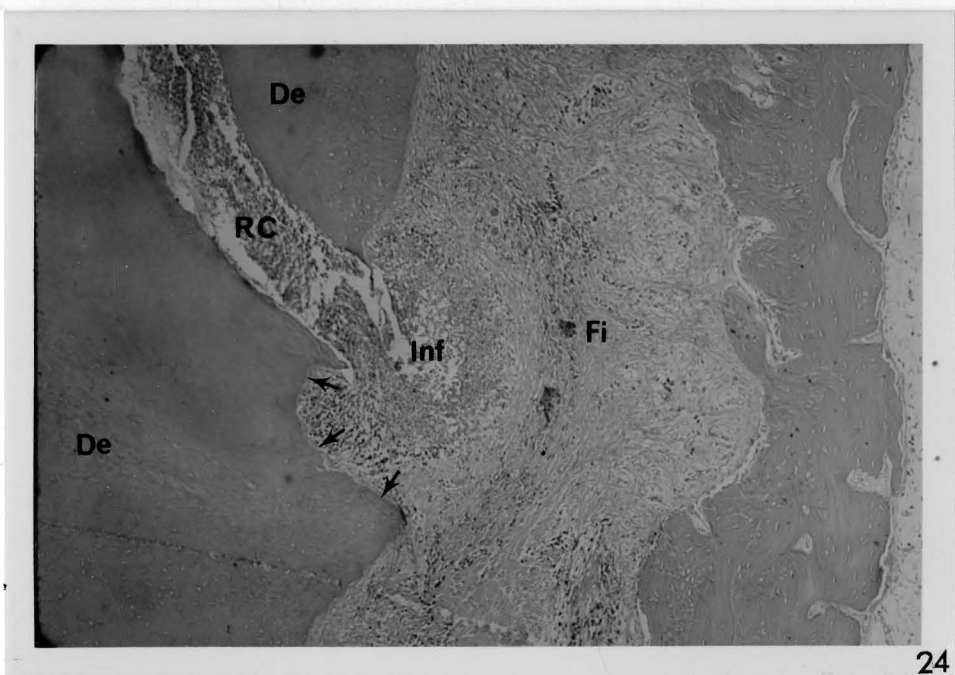


Figure 23: Ninety day specimen (monkey 4) of implanted maxillary defect. Higher magnification of area seen in Fig. 22. Note DABM fragments (arrows) totally incorporated into mature bone trabeculae. Several lacunae within the incorporated fragments appear to have been repopulated with host cells (double arrows). (Masson's trichrome connective tissue stain. Original magnification, X100.)



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Figure 24: Specimen showing control lesion at apex of tooth #21 (monkey 1), 135 days after pulpal inoculation. Note chronic inflammation (Inf) extending into the root canal (RC), resorptive defect (arrows) extending into the root dentin (De), and dense fibrous connective tissue capsule (Fi). (Hematoxylin and eosin stain. Original magnification, X40.)



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Figure 25: Specimen showing control lesion at root apex (R) of tooth #28 (monkey 2), 169 days after pulpal inoculation. Note chronic inflammatory area (Inf) surrounded by a dense fibrous connective tissue capsule (Fi). (van Gieson's collagen stain. Original magnification, X40.)

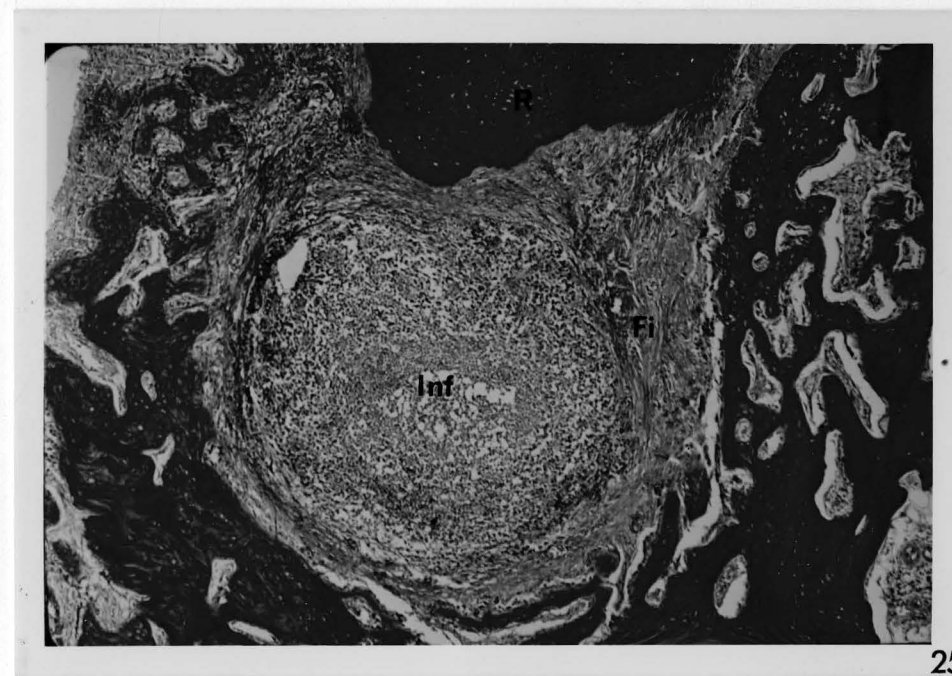


Figure 26:

Pre-operative radiograph of the maxillary anterior region of the Rhesus monkey, exhibiting normal anatomic relationship of structures. Note the extreme proximity of the apices of the central and lateral incisors to each other and to the nasal cavity. (Magnification, X2.5.)



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Figure 27:

Pre-operative radiograph of the mandibular anterior region of the Rhesus monkey, exhibiting normal anatomic relationship of structures. Note how the roots of the four incisors curve toward each other, with their apices being very close together. Note also, the proximity of the lateral incisor apices to the mesial surface of the roots of the canines. (Magnification, X2.5.)



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Figure 28:

Radiograph of maxillary anterior region of monkey 3, immediately after completion of root canal therapy (98 days after pulpal inoculation). Periapical radiolucent areas are visible at the apices of the roots of the incisors. (Magnification, X2.5.)

Figure 29:

Radiograph of maxillary anterior region of monkey 3, at time of sacrifice (28 days after periapical surgery). DABM was implanted into the defect at the apices of #9 and 10 (right). Note little change from Fig. 28. (Magnification, X2.5.)



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